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Volume 42
1946

PUBLISHERS
AMERICAN MEDICAL ASSOCIATION
CHICAGO, ILL

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TUMOR OF TACTILE END ORGANS

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THE OCCURRENCE of a tumor consisting of tactile end organs is rare. Foot,¹ in his review of tumors of the peripheral nerves in 1940, said about the end organs "That there should be tumors of these is to be expected." Such a possibility has been confirmed in the present case in which a tumor was found which in its tinctorial properties and in its structure differs from those previously described as far as I am aware of them.

Thoma² in 1894 described a neuroma of a pacinian corpuscle, an observation referred to by Borst³ in 1902 and by Ewing⁴ in 1940. Most likely, also, Axman⁵ observed hypertrophy of a similar organ. In a patient suffering from plexiform neurofibroma, Brøgli⁵ demonstrated many small tumors of connective tissue with three to eight tactile corpuscles. The maximum length of these was 190 microns, with a maximum width of 100 microns. They were surrounded by one to three layers of connective tissue, which sometimes increased in number from ten to thirty. Some regions showed cancer of the fibromatous tissue. A nearly identical case was published by Scherer,⁶ who found many polyhedral tactile corpuscles. These appeared as lamellated organs of folded tactile cells surrounded by a single layer of connective tissue. In the intracorpuseular cells the nuclei were situated peripherally. Many corpuscles were gathered in groups the size of a bean, attached to the main neurofibromatous tumor.

From the Institute of Pathology (Prof L. Kreyberg, M.D., director) and the Institute of Anatomy (Prof K. E. Schreiner, M.D., director), University of Oslo.

1 Foot, N. C. Arch Path **30** 772, 1940.

2 Thoma, cited by Brøgli,⁵ Foot¹ and Scherer⁶.

3 Cited by Brøgli.⁵

4 Ewing, cited by Foot¹.

5 Brøgli, M. Frankfurt Ztschr f Path **41** 595, 1931.

6 Scherer, H. J. Virchows Arch f path Anat **292** 479, 1934.

7 Masson, P. (a) Lyon chir **21** 257, 1924, (b) Ann d'anat path **1** 3, 1924, (c) **3** 417 and 657, 1926, (d) Arch per le sc med **50** 47, 1927, (e) Am J Path **4** 181, 1928, (f) in Penfield,²² vol 3, p 1095, (g) Bull Soc franç de dermat et syph **42** 1112 and (h) 1278, 1935.

After the brilliant studies of Masson⁷ during the last two decades, the relationship between many tumor types and tactile end organs has been confirmed. Studying the glomic tumor or the *glomus neuromyo-arteriel* in the years 1924 to 1927, he demonstrated in it vessels, smooth muscles and nerve elements, such as cells of Schwann and epithelioid cells. He pointed out the similarity of structure between this type of tumor and the normal glomus in the subepidermal connective tissue. About the same time (1926) Masson observed in the nevus tumors a hyperplasia of the cells of Schwann which led to the formation of *lamés foliacées* or Verocay bodies. These could be interpreted as folded lamellas of tactile cells in the Meissner bodies. The same tumor could also contain areas with a neoplastic reaction of the tactile disks of the epidermis. Masson^{7e} mentioned that most likely the neurofibromatous tumor itself developed from cells of Schwann. In this tumor the cells may be able to differentiate into Verocay bodies and tactile end organs of Meissner.

REPORT OF A CASE

A man 50 years of age had, on the medioulnar side of his left second finger, a congenital tumor. The medial side of the two outer phalanges showed an oval protrusion. On the ulnar side of the tip of the same finger an exostosis was observed. The man could work without any difficulty and did not complain of any pains. In the last two to three years the tumor had grown somewhat larger. The whole tumor with the bone exostosis was completely removed by Dr. Herman G. Gade, temporary chief of Surgical Department A of the University Clinic, Oslo.

Dr. Gade gave me permission to use the clinical records.

There were no postoperative complications. On removal the tumor seemed to be a common perineural fibroma or neurofibroblastoma. The patient did not show traces of any other tumor, and inquiry disclosed no neurofibromatosis in his family.

When the tumor was examined in the Institute of Pathology, it appeared encapsulated, hard, fibromatous, whitish, with the total length 6 cm., the greatest width 3 cm. and the thickness 1.5 cm. The shape was oblong, and the surface showed many small rounded nodules, one of them longer than the others. On one side the tumor was attached to a small flat piece of bone.

Microscopic Examination—Microscopic examination revealed masses of connective tissue. The tissue was looser at the periphery with many large vessels and a pacinian corpuscle. In the central parts of the fibrous tissue were a few small nodules, which differed considerably in size. They showed a peculiar structure and were well delimited without any tendency to infiltrate the surrounding tissue. In the center of the tumor the connective tissue formed a rounded fibrous mass, whereas in the more peripheral parts it extended into many small processes, which were separated by fat, vessels and a loose connective tissue. In figure 1 individual nodules may be recognized surrounded by concentrically arranged, parallel collagenous fibrils. The breadth of this capsule-like structure is greatest in the largest nodules. Extending from the larger external capsule were fine septums of connective tissue which divided the nodules into smaller parts or fields and invaded them completely. Finally, the collagenous fibrils enclosed the indi-

vidual small rounded and oval corpuscles which constituted the nodules of the tumor (fig 2) Thus a striking similarity between these capsules, septums and whirls of connective tissue and the different sheaths of a peripheral nerve was disclosed (fig 1)

The well delimited nodules were composed of small groups of oval, rounded or more irregularly shaped corpuscles (fig 2) Peripherally in the corpuscles

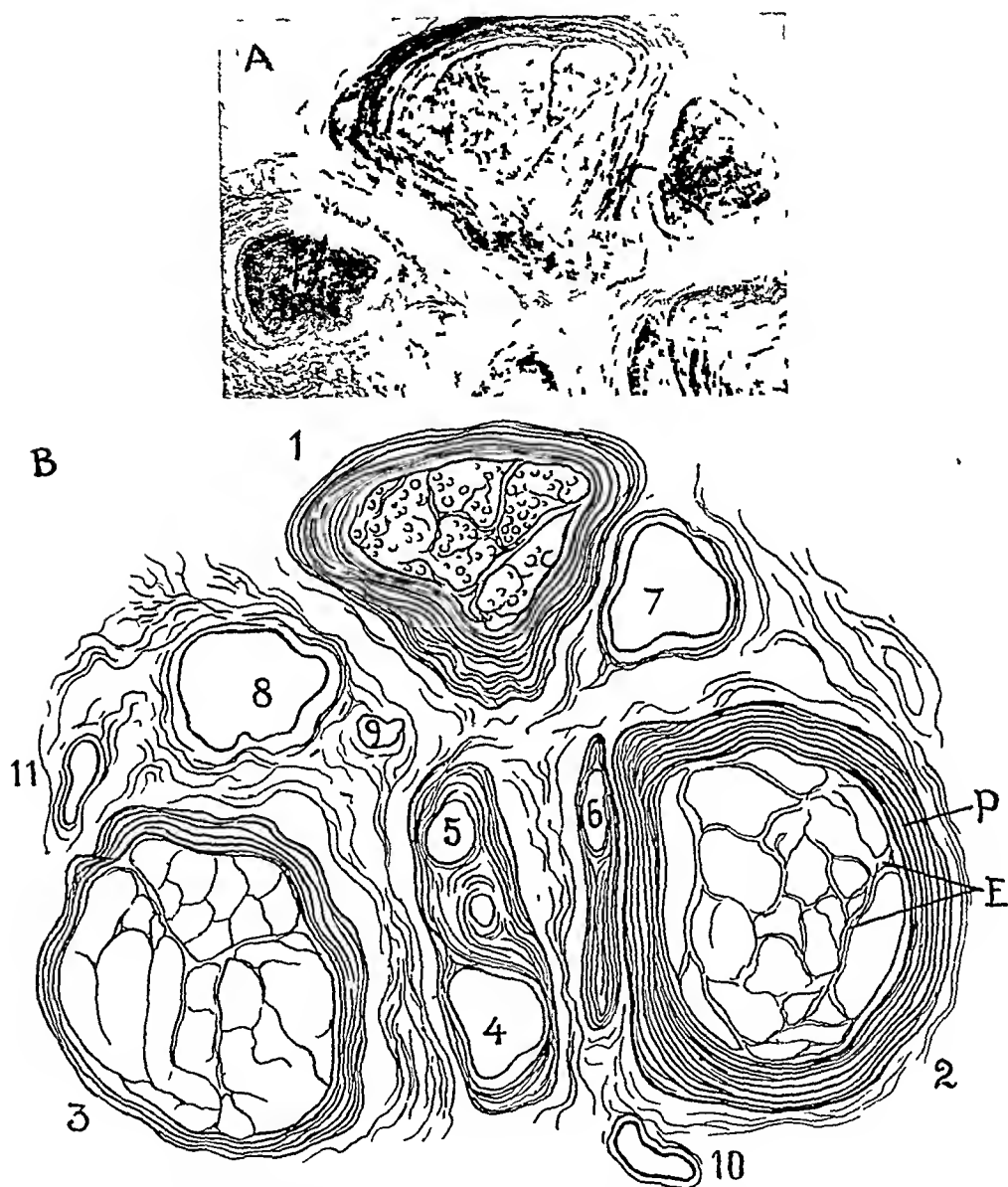


Fig 1—*A*, conglomerates of tactile end organs of different sizes from a part of the whole section represented in *B* Note sheaths of connective tissue dividing the nodules into bundles Van Gieson stain, green filter, $\times 16\frac{1}{3}$

B, semischematic drawing of *A* An extension of the tumor is composed of nodules (1 to 11), which are surrounded by a sheath more or less rich in connective tissue, resembling a perineurial sheath (*P*) Septums of connective tissue like endoneurial sheath (*E*) divide the nodules into many bundles These contain tactile end organs, which individually are encircled by collagenous fibrils of connective tissue from the endoneurial-like sheath The detailed structure is shown in a few nodules only

the cells were flattened and curved with their cytoplasm showing affinity for tri-nitrophenol in Van Gieson-stained sections. The centrally placed nuclei of these cells were oblong and somewhat smaller than the nuclei of the surrounding fibroblasts (fig 3)

Some of the corpuscles were very large, with several layers of capsule cells, which were more densely packed toward the periphery (fig 2B). Some of the corpuscles resembled lamellated tactile bodies

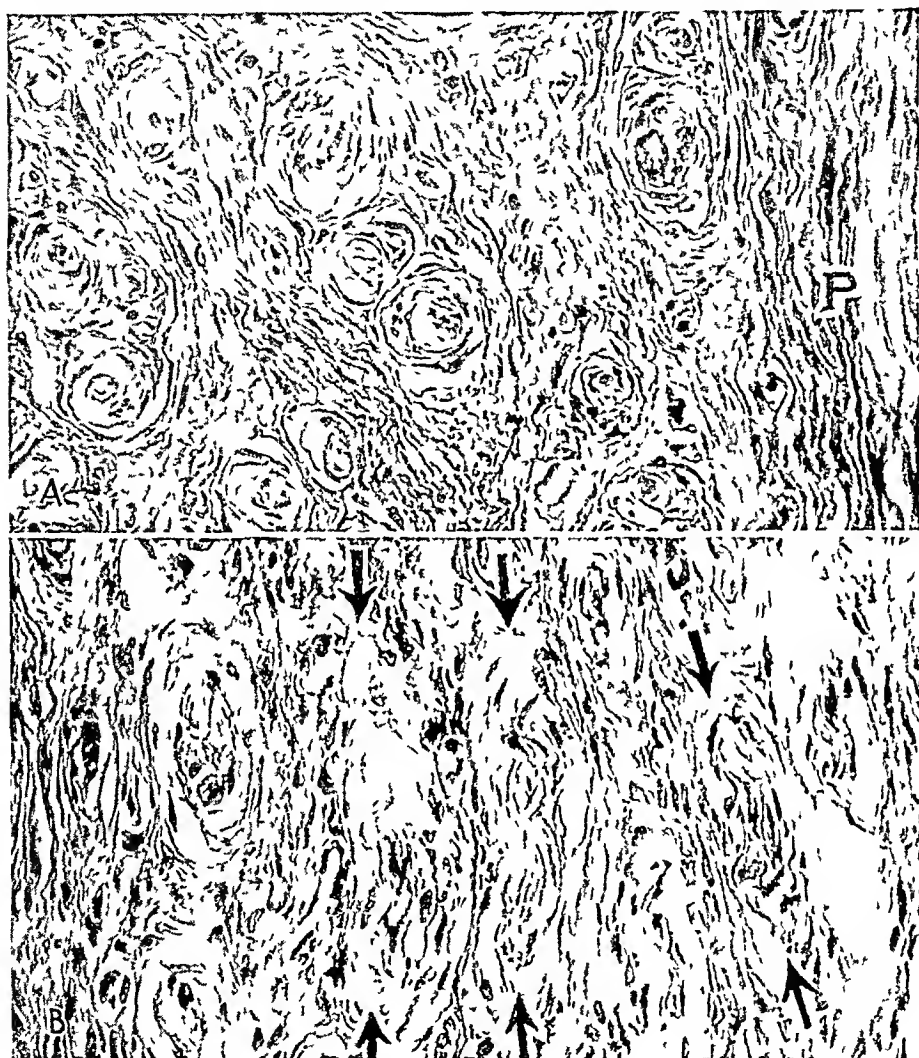


Fig 2—*A*, rounded tactile end organs of different sizes surrounded by collagenous fibrils of connective tissue constituting the perineural (*P*) and endoneural sheaths. Mallory-Azan stain, $\times 312$

B, elongated end organs with the same structural details as in the rounded types. The poles are marked by arrows. Van Gieson stain, green filter, $\times 312$

The shape and the size were not always easy to distinguish, especially in folded types. In some instances quite minute corpuscles were situated close together in small rows, encapsulated by a common layer of elongated capsule cells. In the longest corpuscles short septums were often seen to project from the capsule

into the central core. Scattered among these relatively big corpuscles small ones were found, the capsules of which consisted of a single layer of cells with thin, fibril-like trinitrophenolophilic cytoplasm. In some of the nodules these minor types of corpuscles were gathered in small groups ("primary bundles") or chiefly composed the nodules of the tumor (fig 1 *B*, nodules 7, 8, 9, 10 and 11).

In order to get a more precise idea of the size of the corpuscles, they were measured in a Van Gieson-stained section, illustrated in figure 1 *A* and figure 2 *B*. In this section the contour of the corpuscles was distinct, owing to the affinity of the capsule for trinitrophenol, which gave a precise contrast to the fuchsinophilic connective tissue. The longest and shortest axes of the corpuscles with their capsule cells were measured by means of an ocular micrometer.

The number of corpuscles in the different nodules varied greatly. The nodules labeled 1 to 11 in figure 1 *B* contained the following numbers of corpuscles: 1—331, 2—380, 3—364, 4—56, 5 and 6—6, 9—96, 8—37, 9 and 10—14, 11—3, or a total of 1,287 corpuscles.

The longest axes varied between 81 and 226.8 microns. The mean value (\bar{M}) was 43.4 microns, with a standard deviation (σ) of ± 27.3 microns. As to the shortest axes, the values lay between 5.2 and 11.34 microns, with the mean (\bar{M}) 26.3 microns and the standard deviation (σ) ± 17.6 microns.

The central part of the corpuscles consisted of some few cells with finely vacuolated cytoplasm and fibrils of connective tissue, which in Van Gieson-stained sections showed a trinitrophenolophilic and fuchsinophilic character, respectively. The number of these cells varied a little, and the cells were mostly placed in the central part of the corpuscle (figs 3 and 4). Between the collagenous fibrils appeared clear spaces and clefts, the nature and the development of which were not easy to understand. Sudan-stained sections made clear that the corpuscles were free from lipoids. In silver-impregnated sections the centrally placed cells stood out as individual elements with a distinct nucleus and membrane (fig 3 *F* and *G*, fig 4 *a*, *b* and *d*). The cytoplasm did not contain any pigment.

The demonstration of silver-impregnated nerve fibers within the corpuscles and in the connective tissue was of the greatest importance for the classification of the tumor. These nerve fibers penetrated the capsule at one pole of the corpuscle (*x* in fig 3 *B*). Both the number of nerves and their caliber varied. Many thin parallel fibrils or one thick fiber accompanied on each side by thinner fibrils could be packed so closely together as to resemble a tactile nerve disk. The main nerve fiber was mostly situated in the axial part of the corpuscles (fig 3 *A*, *r* in *B*, and *I*, fig 4), but could also run circularly along the periphery (fig 3 *D* and *E*). Between the thick and thin nerve fibers appeared thin anastomotic fibrils. These thin fibrils constituted a wide-meshed fibrillar network (fig 4 *e*).

When the main axial nerves from the periphery were examined, characteristic findings were noted in most of the corpuscles. The nerve lay on the axial side of the nucleus of the tactile cell and showed an arborization or a periterminal network (figs 3 *C* and 4 *b*). This partly covered the nucleus on the side opposite that facing the point where the nerve entered the corpuscle (fig 3 *A*, *D*, *F* and *H*). The periterminal thin fibrillar network of the nerve lay just outside the nucleus and distinctly within the cytoplasm (fig 3 *C* and *F* to be compared with the schematic figs 4 *a* and *b* and 3 *A*). As seen from the same figures, the extension of the periterminal network could vary somewhat, the meshes could be wider or smaller, and in some specimens only a black impregnated mass was observed.

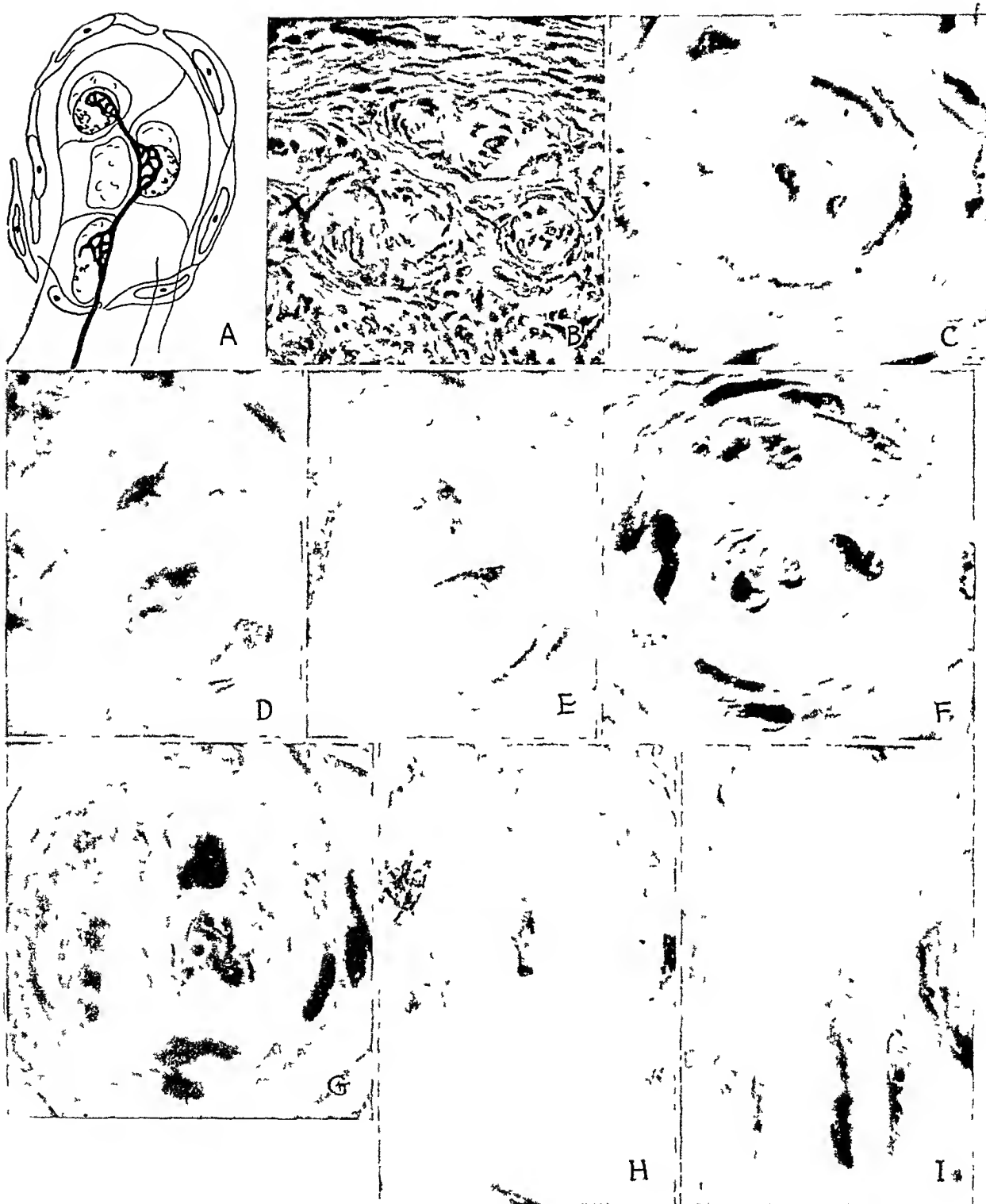


Figure 3

(See legend on opposite page)

The nerve passing the tactile cell membrane showed striking differences in its silver-impregnated appearance. Intracellularly the nerve was somewhat thicker and not quite as smooth as it was outside the tactile cell. This phenomenon was found in the corpuscle illustrated in figure 3 *F* and *G*, but owing to the high magnification used in these photomicrographs, the nerve is not seen in its full extension. In a semischematic drawing the remarkable change of the nerve is illustrated (fig 4 *a* from the same focus).

Another peculiar finding appeared occasionally in oblong corpuscles in which the axial main nerve trunk passed along the nuclei of several tactile cells (fig 3 *I* and the corresponding schematic fig 4 *c*). The nerve then showed a swelling with the endoneural fibrils spread apart. These fibrils covered a part of the nucleus. Along the same nerve were thus seen as many oblong swellings as there were tactile cell nuclei passed.

Other staining methods did not disclose any noteworthy findings, no deposition of lipid granules, no pigments of melanin or iron and, especially, no distinct myelin sheaths.

Summarizing the findings of the anatomic examination, one may say that the tumor consisted of a mass of fibrous connective tissue which surrounded nodules or conglomerates of tactile end organs. In appear-

EXPLANATION OF FIGURE 3

A, schematic drawing of the composition of the tactile end organs as observed in the present tumor. Note the flattened cells (showing affinity for trinitrophenol in Van Gieson-stained sections), which encapsulate the corelike central part with nucleated tactile cells. The nerve fiber covers each nucleus with a network situated within the cytoplasm. Thin anastomosing fibrils connect the nerve fibers and fibrils of different calibers. The collagenous fibrils are not taken into consideration.

B, comparatively clear corpuscles with impregnated nerves of varying caliber, distinctly seen in *r* but only faintly in *y*. Silver impregnation after Bodian, yellow filter, $\times 286$.

C, centrally placed, fine-meshed, black-impregnated, periterminal network covering part of an indistinctly seen nucleus, a small triangular part of the cytoplasm of a grayish tone is seen on the right. At the lower right is seen a similar hooklike part of a terminal ending. Compare with figure 4 *b*. Silver impregnation after Bodian, yellow filter, $\times 1246.5$.

D, black masses of periterminal network covering part of a nucleus near the lower right corner. The nerves continue as parallel curved bands along the periphery of the corpuscle, also visible in *E*.

E, same field as *D* with moderately changed focus. Centrally placed is a large nucleus of a tactile cell with an indistinct network on the upper right side. Compare with figure 4 *d*. Silver impregnation after Bodian, yellow filter, $\times 1246.5$.

F, dark budlike periterminal network near the centrally placed nuclei of the tactile cells, at the lower left.

G, same field as *F* with changed focus, showing the gathering of the branches from the terminal network into a distinct lightly curved nerve just below a nucleus with a definite nucleolus. (For proper comparison with *F*, *G* should be turned 90 degrees to the left.) The nerve passes outside the nuclear membrane, and within the cytoplasm the contour of its membrane appears more vaguely. Compare with figure 4 *a*. Silver impregnation after Bodian, yellow filter, $\times 1246.5$.

H, terminal ending of the nerve at the top of an indistinctly seen central nucleus. From below thin fibrils bend toward the left side of the nucleus. For proper comparison with *I*, *H* should be turned 180 degrees.

I, continuation of the nerve from the budlike termination in *H* with the fibrils spreading over nuclei of the tactile cells which the nerve is to pass. Compare with figure 4 *c*. Silver impregnation after Bodian, yellow filter, $\times 1246.5$.

ance this highly differentiated tumor resembled in many ways enlarged nerves with different layers of connective tissue or sheaths comparable to the epineurium, perineurium and endoneurium of the peripheral nerves. Each end organ was surrounded by capsule cells.

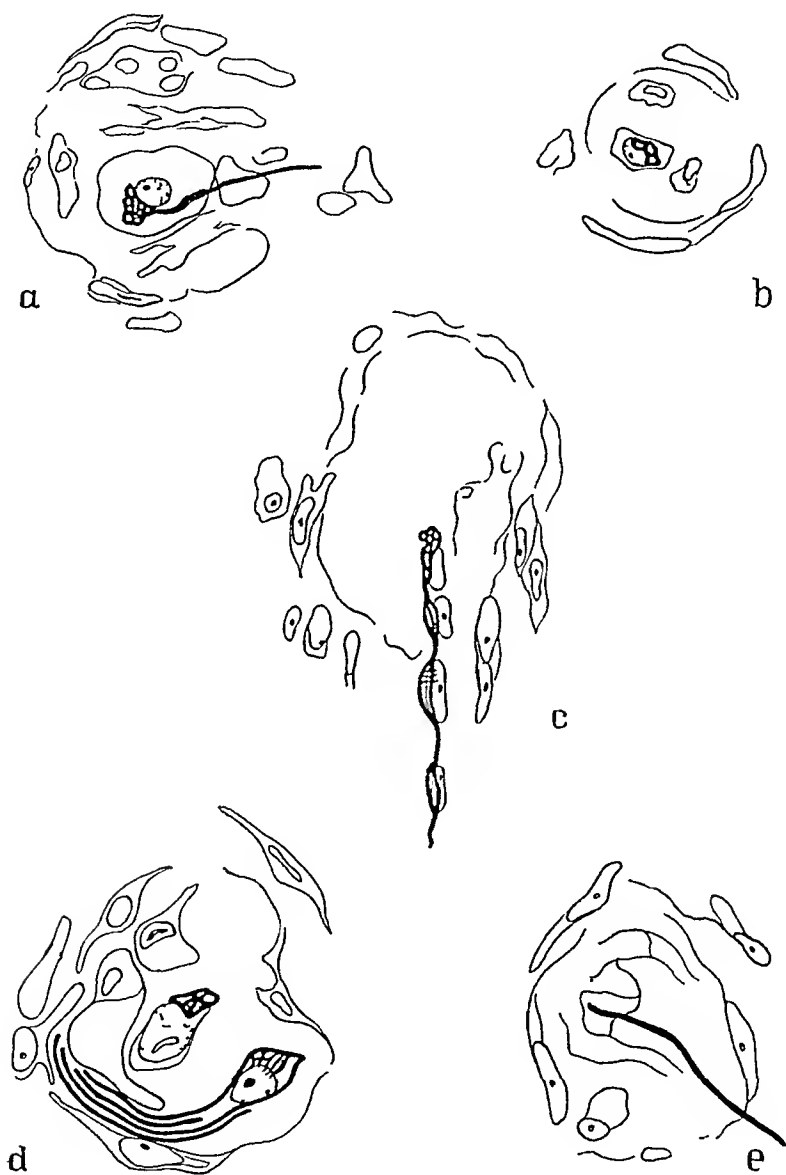


Fig 4—Schematic drawings of the corpuscles as they were seen in the fields illustrated in the photomicrographs. *a* from figure 3 *F*, *b* from figure 3 *C*, *c* from figure 3 *H*, *d* from figure 3 *D* and *e* from a photomicrograph not reproduced. See explanation in the text.

which in their tinctorial characteristics might be identified as Schwann cells or derivatives from the ectoderm. The central part of each corpuscle showed a corelike structure with collagenous fibrils taking

the fuchsin of the Van Gieson stain or the blue tone of the Mallory stain. In silver-impregnated sections nerve fibers were seen in the intercorpuscular connective tissue as well as in the corpuscles themselves. The fibers were of different caliber, and as more or less compact parallel threads they followed an irregular course through the axial part of the corpuscle or even circularly along the periphery. The main nerve ran along the axial side of the elongated tactile cell nucleus and showed intracellularly a swelling or a terminal arborization which partially covered the aforementioned nucleus. A wide-meshed network of thin fibrils connected the thin and thick nerve fibers.

COMMENT

Comparing the present case with the previously published cases, no appreciable similarity could be proved. The neoplastic or tumor-like hypertrophy of end organs described by Thoma,² Axmann,³ Brøgli,⁵ Scherer⁶ and Masson⁷ showed another structure than the present case.

One may only point to the peripheral localization of the intra-corpuscular nuclei and the onion-like lamellation of the corpuscle, neither of which was characteristically present in the case described here. Scherer,⁶ in his case, described a greenish yellow color of some collagenous fibrils found in the core, in contradiction to the red tone of the same parts in my Van Gieson-stained sections. In the two observations which are most similar to the present case, viz., Brøgli's case⁵ and Scherer's case,⁶ silver-impregnating methods were not used.

The present tumor contained multiple tactile end organs or corpuscles. When these were examined for details, the number of nerves, their course and their caliber showed great variations. It was therefore not possible to demonstrate one corpuscle typical for the whole tumor. A rigorous classification of the different types of corpuscles which constituted the tumor was not carried out, owing to the fact that a thorough comparison with the many descriptions already published on normal tactile corpuscles, was impossible (Dogiel,⁸ Smirnow,⁹ Timofeyeff,¹⁰ Michailow¹¹ and others). These studies have revealed a great number of normal anatomic facts. However, among the older investigations one may find that a new name has been given to a corpuscle when only a slight variation was observed. Most histologists nowadays are more inclined to distinguish only between a few groups of end organs with typical structure, since the smaller divergencies

8 Dogiel, A. S. *Arch f mikr Anat* **41** 62, 1893, **59** 1, 1902, **64** 173, 1904

9 Smirnow, A. *Internat Monatschr f Anat u Physiol* **10** 241, 1893

10 Timofeyeff, D. A. *Anat Anz* **11** 44, 1895, *Ergebn d Anat u Entwicklungsgesch* **7** 627, 1897

11 Michailow, S. *Anat Anz* **1** 81, 1907, *Anat Hefte* **41** 495, 1910

should be regarded as the expression of individual variations (van de Velde,¹² Støhr,¹³ Boeke^{14a})

The structure of the tactile end organs in the present case confirmed many observations as to normal anatomic features, especially that as to the intracellular localization of the periterminal network (compare van de Velde¹² and Boeke^{14a}) The changes noted in the physicochemical property of the nerve as it passes the tactile cell membrane and as demonstrated in silver-impregnated sections cannot so far be explained

As in the normal tactile corpuscles, the nerve fibers of different caliber were associated in a common network The meshes contained the tactile cells and the collagenous fibrils Apart from the remarks of Scherer,⁶ no special reference to the tinctorial properties of this central part of the corpuscle was found in the other papers Most students arrived at the conclusion that the central corpuscular structure was of a collagenous nature Boeke^{14b} mentioned a network of nerve fibers built on "a connective tissue bed" Further discussion of the development of the fuchsinophilic fibrils within the tactile bodies would take one far away from the scope of the present investigation Many possibilities could be envisaged—for instance, the result of reactive changes, the product of malformation and the peculiar property of the lemnoblastic cells Uncertainty also arises from the fact that one cannot determine the true nature of the tissue elements or their genetic association from present histologic staining methods (Scherer¹⁵)

The present tumor, which contained well developed tactile corpuscles, did not give any subjective sensation to the patient In other cases of hypertrophy of similar organs, however, as in the glomic tumor of Masson,^{7a} the patient usually does not complain of pains, or the pains are present only for a short period Masson expressed the belief that a glomic tumor may elicit pain during its growth by compression of a pacinian corpuscle in the neighborhood The tumor itself does not seem to have any receptive power In the present case the deep situation of the tumor and the surrounding masses of connective tissue may have prevented the corpuscles from receiving their proper stimuli From the anatomic study it is impossible to state which impulses the corpuscles in casu may respond to (Støhr¹³, Boeke^{14a}, Barcroft¹⁶), although physiologic experiments have demonstrated convincingly that

12 van de Velde, E Internat Monatschr f Anat u Physiol **26** 225, 1909

13 Støhr, P, in von Mollendorf, W Handbuch der mikroskopischen Anatomie der Menschen, Berlin, Julius Springer, 1928, vol 4, pt 1, pp 143-201 and 202-264

14 Boeke, J, in Penfield,²² vol 1, (a) p 241, (b) p 300

15 Scherer, H J Virchows Arch f path Anat **291** 321, 1933, footnote 6

16 Barcroft, cited by Bazett¹⁸

touch, pain, cold and heat act on different corpuscles (Bronk¹⁷, Bazett¹⁸, Woollard¹⁹, Lewis²⁰)

As for the genesis of the tumor, one may believe that the normal development has been impeded. The nerve fibers, as well as the lemnoblastic cells, destined for the formation of the capsule cells of the tactile organs, have not reached their final localization. A similar theory of a faulty organization of embryonal tissue was emphasized in the development of other neurogenic tumors (Pick and Bielschowsky,²¹ Penfield²² and others). The same point of view was favored by the nodular structure of this tumor and the structure of the connective tissue, which resembled the sheaths of peripheral nerves²³

SUMMARY

From the finger of a man 50 years old a tumor was removed, consisting of conglomerates of highly differentiated tactile end organs with silver-impregnable nerve fibers, which were encapsulated in great masses of connective tissue. The connective tissue consisted of collagenous fibrils arranged as in the different sheaths of the peripheral nerves.

The most plausible pathogenetic factor appears to be an inhibitory effect on the development of the nerve endings and their tactile corpuscles.

17 Bronk, D. W. *A Research Nerv & Ment Dis*, Proc **15** 60, 1935

18 Bazett, H. C. *A Research Nerv & Ment Dis*, Proc **15** 83, 1935

19 Woollard, H. H. *Brain* **58** 352, 1935

20 Lewis, T. *Pain*, New York, The Macmillan Company, 1942

21 Pick, L., and Bielschowsky, M. *Ztschr f d ges Neurol u Psychiat* **6** 391, 1911

22 Penfield, W. *Cytology and Cellular Pathology of the Nervous System*, New York, Paul B. Hoeber, Inc., 1932, vol. 3, p. 953

23 N. C. Foot has briefly described, in a neurofibroma removed from the scalp, a nodule closely resembling a pacinian corpuscle which appears to be similar to the first observed hypertrophic type (*Pathology in Surgery*, Philadelphia, J. B. Lippincott Company, 1945. This book became available in Norway after the present article had been submitted for publication).

PATHOLOGIC ASPECTS OF ATMOSPHERIC BLAST INJURIES IN MAN

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DURING World War II, in many countries both the military and the civilian population were exposed to the destructive effects of the detonation of high explosives. As a result, blast injury assumed great importance, and its study was the immediate concern of a number of investigators on the home fronts and in the battle zones. Interest in the problem was particularly great in the Armed Forces, since the Navy had to deal with immersion blast and the Army with atmospheric blast. Though the incidence of injury due to blast undoubtedly was high among military personnel, the number of recorded detailed clinico-pathologic observations on soldiers dying from this type of injury was small.

Thus in the files of the Army Institute of Pathology there are available the records and the material of but 11 cases in which the information concerning the circumstances of the blast is adequate and the lesions are unmistakable. Although the series is small, it is of importance, for hitherto most observations on the pathologic aspects of injuries due to atmospheric blast have been based on animal experimentation.

CLINICAL NOTES

CASES 1 to 6—The 6 patients will be considered as a group, since fatal injuries in all resulted from the same accident. A heavy bomber, returning from a mission, crash-landed in an ammunition dump in which there was a large quantity of high explosive (trinitrotoluene). The plane immediately burst into flames. Soldiers stationed at the dump and at installations nearby ran to the scene of the crash to rescue the crew from the burning plane. Suddenly a terrific explosion occurred, and an unknown number of soldiers were caught in it. Six who survived the immediate effects of the blast were evacuated to an Army hospital, where they arrived less than an hour after the explosion.

At entry most of the patients showed various degrees of shock and restlessness, 1 was stuporous. A bloody, frothy fluid filled the mouth and the nose, respirations were labored, both exposed and unexposed parts were burned, and widespread punctate hemorrhagic lesions showed where particles of debris had been forcibly

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driven into the skin. There were no significant surface or penetrating wounds and no fractures of bones. The lungs revealed severe bilateral pulmonary edema, at no time were clinical signs of consolidation evident.

Treatment of the burned and shocked patients included the usual supportive measures: injection of plasma, administration of morphine and application of pressure bandages to the affected parts. Serum albumin was administered to 1 patient. Penicillin in the amount of 40,000 units was given to all patients with burns every two hours. Blood pressure readings were recorded for 4 patients, in 3 the low pressure noted on admission was successfully combated only to fall terminally, in the fourth the shock level of the pressure persisted. In most instances suction had to be employed to clear the mouth and the upper respiratory passages of the bloody, frothy fluid. Dyspnea and pulmonary edema persisted, the restlessness of 2 patients changed to excitability, irrationality and mania. Death was attributed to respiratory failure and shock. The periods of survival are recorded in table 1. A roentgenogram of the chest of the patient who survived seventy-one hours showed "generalized diffuse areas of density throughout both lung fields."

CASE 7—The soldier was in the vicinity of an explosion of stored dynamite. He died ten minutes later. There were no burns, no significant external wounds and no fractures of bones.

CASE 8—The soldier was in a gun emplacement when a 500 pound (227 Kg.) aerial bomb exploded nearby. When he arrived at an evacuation hospital five hours later, he was in profound shock. Despite vigorous antishock therapy and continuous administration of oxygen, the blood pressure continued to fall, and the patient died thirty-six and a half hours after the blast. There were no significant external wounds, no burns and no fractured bones.

CASE 9—The soldier was in a hospital tent when a buzz bomb (V-1) landed and exploded 40 feet (12 meters) away. On admission to the hospital he was unconscious and in shock, his respirations were gasping and irregular, subcutaneous emphysema was apparent, and there was bloody froth in the mouth. With treatment for shock the respirations improved, but subsequently fell to 2 per minute. A roentgenogram of the chest revealed pneumothorax on the left side with mediastinal shift. This condition was successfully relieved by insertion of a flutter valve. Ten hours after the accident the patient regained consciousness, but the respirations and the heart action remained irregular. Fine and coarse rales were diffusely audible in both lungs. A roentgenogram forty-eight hours after the explosion showed changes consistent with blast injuries in both lungs. During the fourth day the patient suddenly became worse, he was disoriented and excitable. All reflexes were hyperactive, but no pathologic reflexes were obtained. The blood pressure was 150 systolic and 75 diastolic. He died on the fifth day, one hundred and twelve hours after the explosion. There were no burns or fractured bones. The only significant external injury was a penetrating wound of the right upper arm, measuring 5 by 3 cm.

CASE 10—The soldier was assisting in the rescue of personnel from a burning plane when bombs which were stored in the plane exploded. The clinical findings were first and second degree burns of the face and of the right upper and lower extremities, shock, hemoptysis, oliguria and rales in both lungs. There were no fractures or significant external wounds. He died in approximately forty-eight hours.

CASE 11—The soldier was caught in an explosion of dynamite. Shock, coma and generalized dusky cyanosis rapidly developed. The cyanosis responded to

continuous administration of oxygen. Suction had to be employed frequently to clear the pharynx of accumulations of bloody fluid. Death occurred eighteen hours after the accident. There were no burns and no fractures, but there were numerous superficial abrasions and contusions of the skin of the face, the chest and the upper and lower extremities.

PATHOLOGIC OBSERVATIONS

The number of hours which elapsed from the time of death until autopsy varied from two to seventeen hours. Six of the 11 patients had surface burns, the degree and the extent of which are indicated in table 1. In 5 the skin of the trunk and occasionally that of the face, the neck and the extremities was peppered with minute hemorrhagic burns or lacerations. These lesions involved the entire thickness of the skin, and a few extended into the superficial subcutaneous tissue. In the center of each a tiny piece of debris or dirt was embedded. In case 3 a laceration, 12 cm. in diameter, and numerous hematomas were present in the scalp of the frontal region, in case 9 a penetrating wound in the right upper arm measured 5 by 3 cm. No significant surface or penetrating wounds were found in the remaining cases, but in the majority there were superficial abrasions or lacerations of the skin over the body (table 1). Inspection and palpation revealed no fractured bones. The muscles of the trunk and the extremities were free of hemorrhage or other signs of injury. The parietal pleura in case 7 showed numerous petechiae, in the remaining cases neither the parietal pleura nor the peritoneum was hemorrhagic or lacerated, and the intercostal vessels were not torn.

The significant lesions in our cases were in the lungs. Since the gross and the microscopic pulmonary changes were fundamentally similar in all the cases of the series they will be described collectively. Lesions of other organs occurred with much less regularity, but in some instances were no less striking than those of the lungs.

Lungs—(a) *Gross Findings*. The lungs were large and heavy, the increase in size and weight affecting both the right and the left lung roughly to the same extent. The average weight of the right lung in this series was 1,020 Gm., that of the left lung 875 Gm. Visible externally were numerous, diffuse, often discrete zones of pleural or subpleural hemorrhages, or both, deep purple-red discolorations, which varied considerably in size, the smallest ones ranging from a few millimeters to 2 cm. in diameter, the largest involving the surface of almost an entire lobe. The hemorrhages showed no tendency to follow the costal markings. In 4 of the 11 cases large amounts of blood had collected beneath the pleura at the interlobar reflections near the hilus. Section of the substance of the lungs revealed many, scattered, solid, dark red, hemorrhagic zones, usually clearly defined, these also exhibited wide variations in size. Occasional large confluent hemorrhages were from 9 to 12 cm. in greatest diameter, and in 1 instance almost an entire lobe was converted into a hemorrhagic mass. The distribution, as well as the size of the hemorrhages, was variable. Some were confined to the subpleural zone, with the remainder of the lobe free of hemorrhage. Other subpleural hemorrhages overlay a relatively nonhemorrhagic layer of parenchyma, with hemorrhage repeated in the underlying tissues. Sometimes the deeper portions of the lung were hemorrhagic while the more superficial parts were not. Finally, some hemorrhages involved the entire thickness of a lobe. In this series of cases the hemorrhages showed no predilection for either lung, a given lobe of either lung or a particular portion of the lobe. Thus the hemorrhages were bilateral, the upper lobes were affected as frequently as the

lower lobes, and the anterior portions were involved as frequently as the posterior portions. In an individual case the hemorrhages were more intense in one lung or in a given portion of a lobe, but in the over-all picture they were bilateral and multilobar and spared no part of the lungs.

The pulmonary parenchyma surrounding the hemorrhages was diffusely pink-red and markedly edematous. In almost every case the dark red hemorrhagic zones stood out in sharp contrast to the surrounding acutely hyperemic and edematous parenchyma. Large amounts of pink, frothy and frankly bloody fluid dripped freely when the lungs were cut. This bloody and frothy fluid also filled the lumens of the bronchioles, bronchi and trachea and was frequently found in the mouth and the nares. The respiratory passages themselves were free of significant changes.

Gross intrapulmonary emphysema and tearing of the lung parenchyma were observed in only 1 case, laceration of the visceral pleura, in none. Pneumothorax

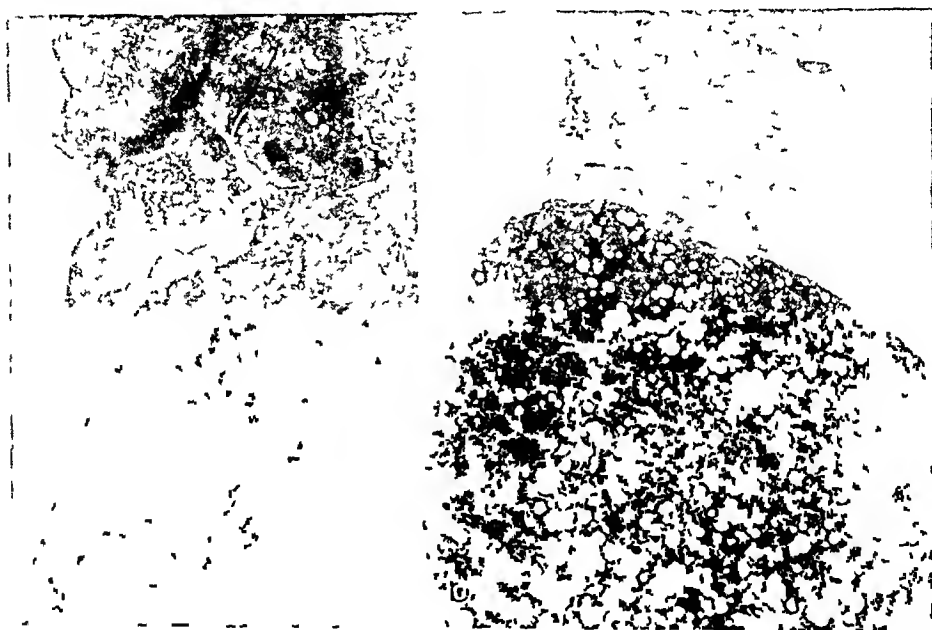


Fig 1—(a) Two contiguous pulmonary lobules. In both there is intense hemorrhage. The interlobular septum is focally hemorrhagic $\times 4$ (Army Institute of Pathology negative 90905). (b) Intense intrapulmonary hemorrhage affecting one entire lobule and completely sparing a contiguous one. The interlobular septum is free of hemorrhage $\times 38$ (Army Institute of Pathology negative 90906).

(unilateral) occurred in 1 case. Free liquid blood was found in the pleural cavities in 7 cases, the hemothorax being bilateral in 6 and unilateral in 1. The amount of blood in the pleural cavity was never in excess of 400 cc, usually between 50 and 125 cc.

(b) Microscopic Changes. The principal lesions were acute hemorrhages, edema and emphysema. The distribution of the hemorrhages was inconstant. Some extended throughout the whole of two or more contiguous lobules (fig 1a), others filled entire lobules but spared adjacent ones (fig 1b), while many involved only parts of single lobules. The hemorrhages varied in intensity as well as in extent. In the profoundly hemorrhagic zones all alveoli, alveolar ducts and respiratory bronchioles and the majority of the larger bronchioles were distended by closely packed erythrocytes. In these areas the alveolar septal

TABLE 1—Summary of Clinical and Postmortem Findings in Eleven Cases of Atmospheric Blast Injuries

| Case | Age | Race | Sex | Survival Time | Surface Burns and/or External Wounds | Weight of Lungs, Gm | Pathologic Findings in Lungs | Pulmonary Fat Embolism | Effects on Other Structures |
|------|-----|------|-----|----------------|---|----------------------|---|------------------------|--|
| 1 | 21 | W | M | 17½ hr | First and second degree, face, chest, upper and lower extremities, (approximately 40 per cent of body surface), skin of trunk peppered with minute hemorrhagic burns | Rt 900 Lt 800 | Intrapulmonary hemorrhages and edema, severe, in all lobes, bilat, most pronounced in lower lobes, severe vesicular and minimal in terstitial emphysema, bilat hemothorax, 50.75 cc | Moderate | Hemorrhages, diffuse, in thymus and peribronchic tissues |
| 2 | 22 | N | M | 8 hr | None | Rt 1 000 Lt 930 | Intrapulmonary hemorrhages and edema, severe, in all lobes, bilat mod vesicular emphysema bronchopneumonia, bilat hemothorax, 300 cc rt, 50 cc lt | Minimal | Hemorrhages, focal, small, in epicardium, myocardium, adrenal gland cortex, kidney |
| 3 | 25 | W | M | Less than 1 hr | Second and third degree, head and face, laceration and hematomas of scalp, skin of neck, trunk and upper extremities peppered with minute hemorrhagic burns, superficial abrasions of lower extremities | Rt 1 000 Lt 1,000 | Intrapulmonary hemorrhages, severe in 4 lobes, edema, severe, in all lobes, bilat, mod vesicular emphysema, bilat hemothorax, 50 100 cc | None observed | Petechiae in epicardium, hemorrhage, diffuse, mod, in leptomeninges |
| 4 | 29 | W | M | 4 hr | Second degree, face, ant part of neck, right side of chest and axilla, upper and lower extremities, above and below elbows and knees, post part of trunk peppered with minute hemorrhagic burns | Rt 1,070 Lt 900 | Intrapulmonary hemorrhages (chiefly subpleural to av depth of 2 cm) and edema, severe, in all lobes, bilat, mod vesicular emphysema bilat hemothorax, 125 cc | None observed | Hemorrhages in mesentery (7 x 9 cm), leptomeninges (diffuse, mod), cortex of rt temporal lobe (focal), petechiae in epicardium |
| 5 | 31 | W | M | 18 hr | First and second degree, face, neck, chest, left forearm, hands, lower extremities | Rt 1,250 Lt 900 | Intrapulmonary hemorrhages (chiefly post) and edema, severe, in all lobes, bilat, mod vesicular emphysema, bronchopneumonia | Moderate | Acute perforation of sigmoid colon peritonitis, hemorrhages, focal, in testis, spleen, oesophageal microscopic perivascular hemorrhages in brain |

| | | | | | | | | | |
|----|----|---|---|--------|---|--------------------------------------|--|---------------|--|
| 6 | 27 | W | M | 71 hr | First and second degree, face, hands and lower extremities, small superficial hemorrhage laceration of upper part of chest | Rt 920 Lt 910 | Intrapulmonary hemorrhages (chiefly subpleural to av depth of 2.3 cm) and edema, mod in all lobes, bilateral vesicular emphysema, antemortem thrombi in mod numbers of pulmonary vessels, hyaline membrane coating of alveolar walls | Moderate | Large hemorrhage in wall of stomach, occasional microscopic hemorrhages in leptomeninges and brain, bilateral perforation of tympanic membranes |
| 7 | 20 | W | M | 10 min | No burns, peppering of minute lacerations in skin of major portion of ant surface of body, penetrating wounds of both eyes, left lower incisors fractured at gingival margin | Rt 1,450 Lt 1,350 | Intrapulmonary hemorrhages and edema, severe, in all lobes, bilateral severe vesicular and interstitial emphysema, small subpleural air blebs visible grossly, much disruption of pulmonary tissues near hilum, bilateral hemothorax, 25-50 cc | None observed | Mediastinal emphysema, small hemorrhages in thymus, parietal pleurae, epicardium, pelvis of kidney |
| 8 | 22 | W | M | 36½ hr | No burns, small contused abrasion of right forearm | Estimated 3 times normal | Intrapulmonary hemorrhages and edema, severe, in all lobes, bilateral bronchopneumonia | Minimal | Hemorrhage in diaphragm in vicinity of esophageal hiatus |
| 9 | 32 | W | M | 112 hr | No burns, small contusions, lacerations and abrasions, superficial, face, right upper extremity, left ankle, penetrating wound of right upper arm, 5 x 3 cm, skin of left ant part of chest studded with minute spots | Rt 1,000 Lt 525 | Intrapulmonary hemorrhages, severe in all lobes of right lung, minimal, in left lung, atelectasis of left lung, left pneumothorax, bilateral hemothorax, 300-400 cc, antemortem thrombi in mod numbers of pulmonary vessels | Minimal | Subcutaneous and mediastinal emphysema, petechiae in omentum and in peritoneal and periaxillary tissues, large tear and massive hemorrhage in cerebellum, punctate hemorrhages in cerebrum, diffuse leptomeningeal hemorrhage in cerebrum and cerebellum |
| 10 | 31 | W | M | 48 hr | First and second degree, face, right hand thigh, knee, superficial abrasions of chest, left arm and leg, hemorrhage in conjunctiva of left eye | Rt 650 Lt 750 (Weights estimated) | Intrapulmonary hemorrhages and edema, moderately severe, bilateral hyaline membrane coating of alveolar walls | Minimal | Petechiae in endocardium epicardium microscopic hemorrhages in myocardium, spleen, focal necroses in spleen, venous thromboses in spleen, heart |
| 11 | 20 | W | M | 18 hr | No burns, hemorrhages in conjunctivas of both eyes, superficial abrasions and contusions of face, chest, upper and lower extremities | Rt 960 Lt 680 | Intrapulmonary hemorrhages and edema, severe, in all lobes, bilateral, mod vesicular emphysema, bronchopneumonia, right hemothorax, 150 cc | Minimal | Internal hemorrhage in left eye, hemorrhages in colon microscopic hemorrhages in myocardium, brain |

markings usually were clearly defined but occasionally had disappeared, leaving a large confluent hemorrhage. In the areas of less intense hemorrhage the alveoli, the alveolar ducts, the respiratory bronchioles and some of the larger bronchioles contained varying amounts of edema fluid in which erythrocytes when present were not closely packed. In the routinely prepared hematoxylin-eosin sections it appeared that many alveolar septums were thickened from hemorrhagic infiltration. By the use of the reticulum stain (Wilder's method) it was found that the majority of these septums were of average thickness and the reticular structure was intact. Actually the hemorrhage had occurred in the alveoli, and the erythrocytes had been displaced peripherally against the septal walls by the large volume of intra-alveolar air. Nevertheless there was unmistakable bleeding into some of the septums. In the foci of intense hemorrhage the peribronchiolar and perivascular supporting tissues, the intralobular septums

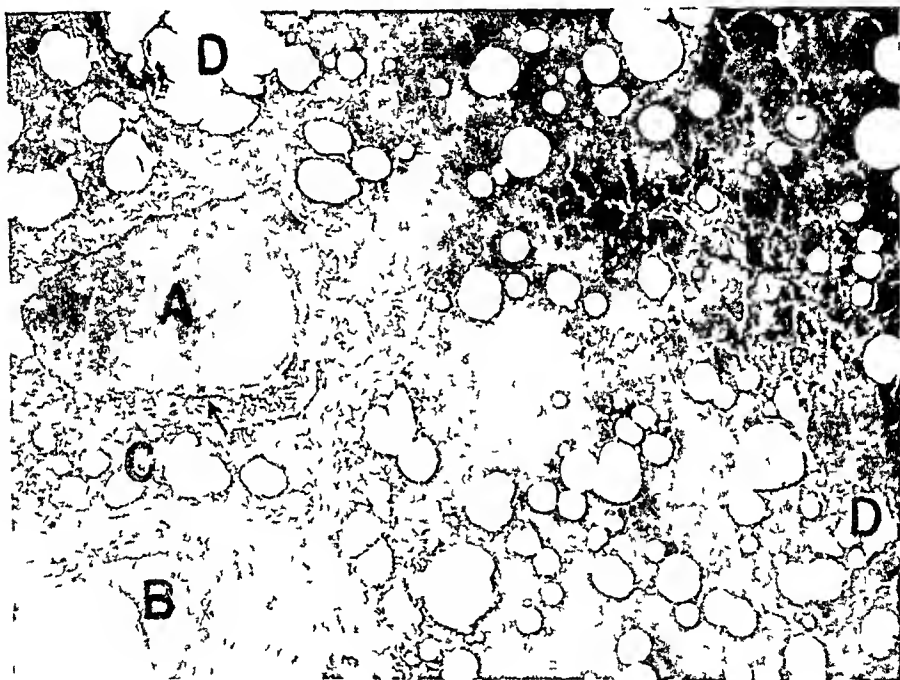


Fig 2—Intense intrapulmonary hemorrhage, severe diffuse vesicular emphysema and focal interstitial emphysema. *A* indicates a bronchiole filled with an erythrocyte cast, *B*, a congested pulmonary vessel, *C*, interstitial emphysema in a bronchovascular septum, *D*, torn and fragmented alveolar septums. The arrow indicates a site of focal ulceration of the bronchiolar epithelium with intense hemorrhagic infiltration of the exposed lamina propria. $\times 254$ (Army Institute of Pathology negative 90899)

and occasionally the interlobular septums were heavily infiltrated with erythrocytes and edema fluid. These tissues were not affected in patches of less intense hemorrhage. In the nonhemorrhagic areas of the lung, intra-alveolar and intra-bronchiolar edema was common.

Severe acute emphysema, noted frequently, was practically always of the vesicular variety, was diffuse and occurred in both the densely and the less intensely hemorrhagic portions of the lung. The alveoli, the alveolar ducts and the respiratory bronchioles were the air passages most constantly involved, the larger bronchioles occasionally. The alveolar septums were at times seen to be

lacerated and fragmented in areas of severe emphysema (fig 2) Although numerous sections of lung were studied, interstitial emphysema was found in only 2 cases One focus, located in a bronchovascular septum, is clearly demonstrated in figure 2

Changes were often observed in the walls of the bronchioles The earliest change appeared to be a loosening or lifting-off of the epithelium from the underlying bronchiolar wall, with displacement or shedding of the epithelium into the lumen The epithelial detachment was partial or complete and sometimes had progressed to fragmentation and disappearance of the epithelium Usually the lamina propria remained attached to the wall, but occasionally it too was partially or completely denuded to expose the internal surface of the bronchiolar musculature At the site of epithelial denudation intense hemorrhagic infiltration of the lamina propria might take place (fig 2) In the space formed when the epithelium separated from the lamina propria, edema fluid often collected, and occasionally the space contained extravasated erythrocytes in moderate numbers



Fig 3—(a) Complete detachment of bronchiolar epithelium Extravasated erythrocytes in moderate numbers have accumulated in the space formed where the epithelium separated from the lamina propria $\times 109$ (Army Institute of Pathology negative 92361) (b) Bronchiole showing detachment of the epithelium, edema fluid in the lumen and edema fluid and groups of erythrocytes in the space formed by the separation of the epithelium and the lamina propria $\times 109$ (Army Institute of Pathology negative 92362)

(fig 3a) In practically every instance in which a subepithelial collection of edema fluid was noted, there was an associated accumulation in the lumen of the bronchiole (fig 3b) At times the lifted-off epithelium was interrupted, establishing continuity between luminal and subepithelial edema Frequently polymorphonuclear leukocytes infiltrated the wall of the bronchiole in minimal to moderate degrees, being usually concentrated in the lamina propria and often completely sparing the muscle (fig 4a) Unless there were secondary purulent bronchiolitis and bronchopneumonia (both were rare) the leukocytes almost always were limited to the wall of the bronchiole and were not in the surrounding parenchyma Occasionally, however, leukocytes in moderate numbers did appear in the peri-

bronchiolar tissue when the lamina propria of the bronchiole was infiltrated (fig 4a) Usually the mural leukocytic infiltration was associated with partial or complete ulceration or loss of epithelium, occasionally it was seen when the epithelium was intact but loosened (fig 4b) In all instances the epithelium appeared to be well preserved and viable The bronchi did not show significant histologic alterations

Engorgement of the pulmonary vessels was constant in the intensely hemorrhagic zones but variable in the remainder of the lung Tears of the walls of

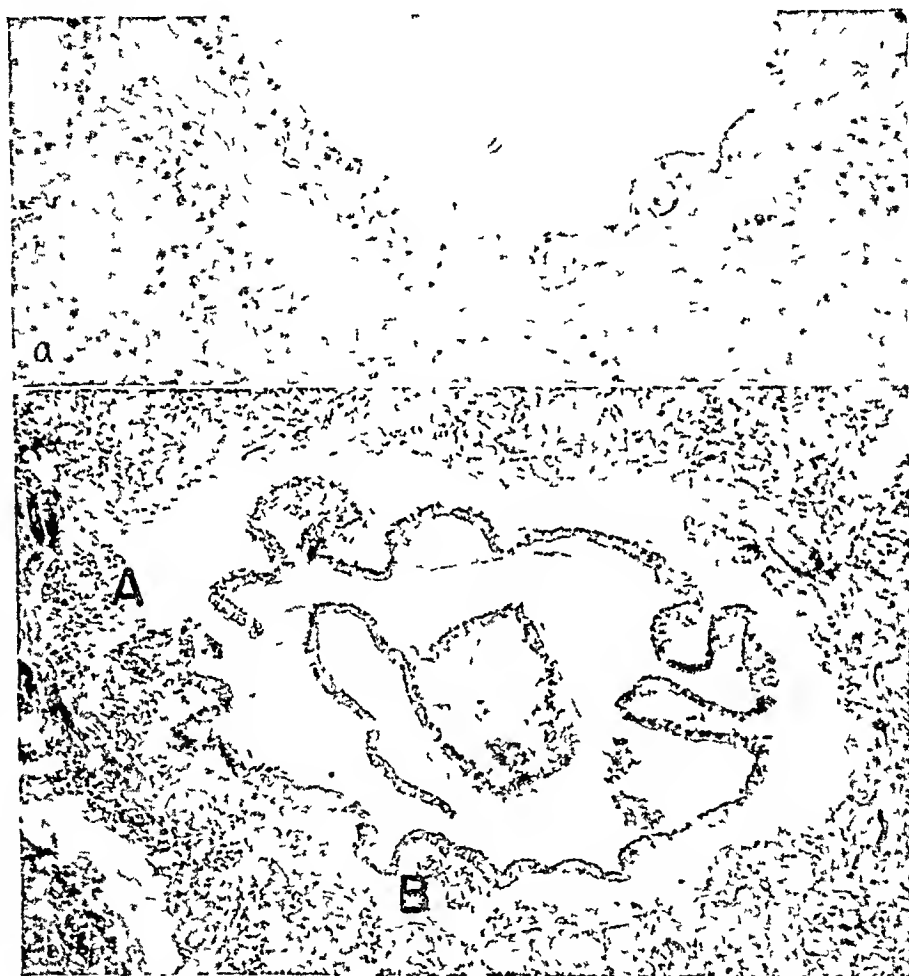


Fig 4—(a) Bronchiole showing complete epithelial ulceration and moderate leukocytic infiltration of the swollen lamina propria The muscularis is free of inflammatory reaction In the peribronchiolar tissue moderate numbers of leukocytes are present $\times 2488$ (Army Institute of Pathology negative 92552) (b) Complete detachment of the bronchiolar epithelium Leukocytes in large numbers have diffusely infiltrated the wall, chiefly the lamina propria The leukocytic infiltration is heaviest at A At B a pool of erythrocytes has accumulated in the subepithelial space The lumen of the bronchiole is free of exudate $\times 888$ (Army Institute of Pathology negative 92363)

pulmonary veins or arteries were never found Antemortem thrombi were seldom noted In the intensely hemorrhagic areas the septal capillaries usually were empty and collapsed (probably due to compression by the intra-alveolar blood),

in contrast to the congestion of the capillaries in sections of the lung which showed edema, moderate hemorrhage or no hemorrhage. As a rule, the lymphatics were dilated and congested.

The visceral pleura was frequently hemorrhagic. The pleural hemorrhage was sometimes directly continuous with a subjacent parenchymal hemorrhage (fig 5a). Sometimes a relatively nonhemorrhagic zone of parenchyma lay between the hemorrhagic pleura and a deep-seated parenchymal hemorrhage (fig 5b), or the lung substance was intensely hemorrhagic and the overlying pleura entirely free of hemorrhage (fig 5c).

Minimal to moderate inflammatory cellular infiltration (principally polymorphonuclear leukocytes and large mononuclear cells) occurred in the hemorrhagic and the nonhemorrhagic portions of the lung in almost every case. A leukocytic response sufficiently heavy to be considered bronchopneumonia was present in 4 cases, with survival times from eight to thirty-six and a half hours.

In all the cases sections of the lungs were stained for fat. Intravascular fat was present in moderate amount in 3 cases and in minimal amount in 5 cases, it was absent in 3.

In case 6 (survival time seventy-one hours) pulmonary hemorrhages, edema and emphysema, though present, were much less pronounced than in the other 10 cases. More conspicuous in this case were (1) a deeply eosinophilic homogeneous hyaline or finely granular material in many of the alveoli and (2) antemortem thrombi in moderate numbers of the pulmonary vessels. This structureless eosinophilic material appeared as sheets filling most of the lumens of the affected alveoli or as thin films or membranes coating the alveolar walls. This phenomenon of the coating of the alveolar walls with a hyaline-like material was also observed in case 10 (survival time forty-eight hours). The material gave a negative staining reaction for fibrin. It probably represented an intra-alveolar transudate which had been displaced peripherally against the alveolar walls. Moderate numbers of large mononuclear cells and a few polymorphonuclear leukocytes were intermixed with some of the sheets of material in the alveoli.

Other Organs and Structures—Changes attributed to blast effect were found in many organs and structures other than the lungs. A summary of the extrapulmonary lesions follows.

(a) Heart. There were subepicardial petechiae in 5 cases, subendocardial petechiae in 1 and microscopic hemorrhages in the myocardium in 3. In the center of one of the myocardial hemorrhages was a small artery occluded by an antemortem thrombus. Microscopic venous thromboses were found in the myocardium in 1 case.

(b) Spleen. In case 5 there were two parenchymal hemorrhages, the larger measuring 2 cm in greatest dimension. The organ was not lacerated, and no thrombi were found. In case 10 microscopic zones of hemorrhages and necroses, as well as thromboses of the veins, especially the trabecular veins, were observed.

(c) Kidneys. In the left kidney in 1 case was a small cortical hemorrhage, 1 cm in diameter. In another case there was a microscopic hemorrhage in the pelvis.

(d) Adrenal Glands. Hyperemia of the cortex, especially in the zona reticularis, was usual. No frank hemorrhages were seen, but in 1 case microscopic hemorrhages were noted in the cortex.

(e) Testes. In 1 case there was a small surface hemorrhage measuring 0.5 to 1 cm in one of the testes. It involved the tunica albuginea and the subjacent parenchyma (fig 6a).

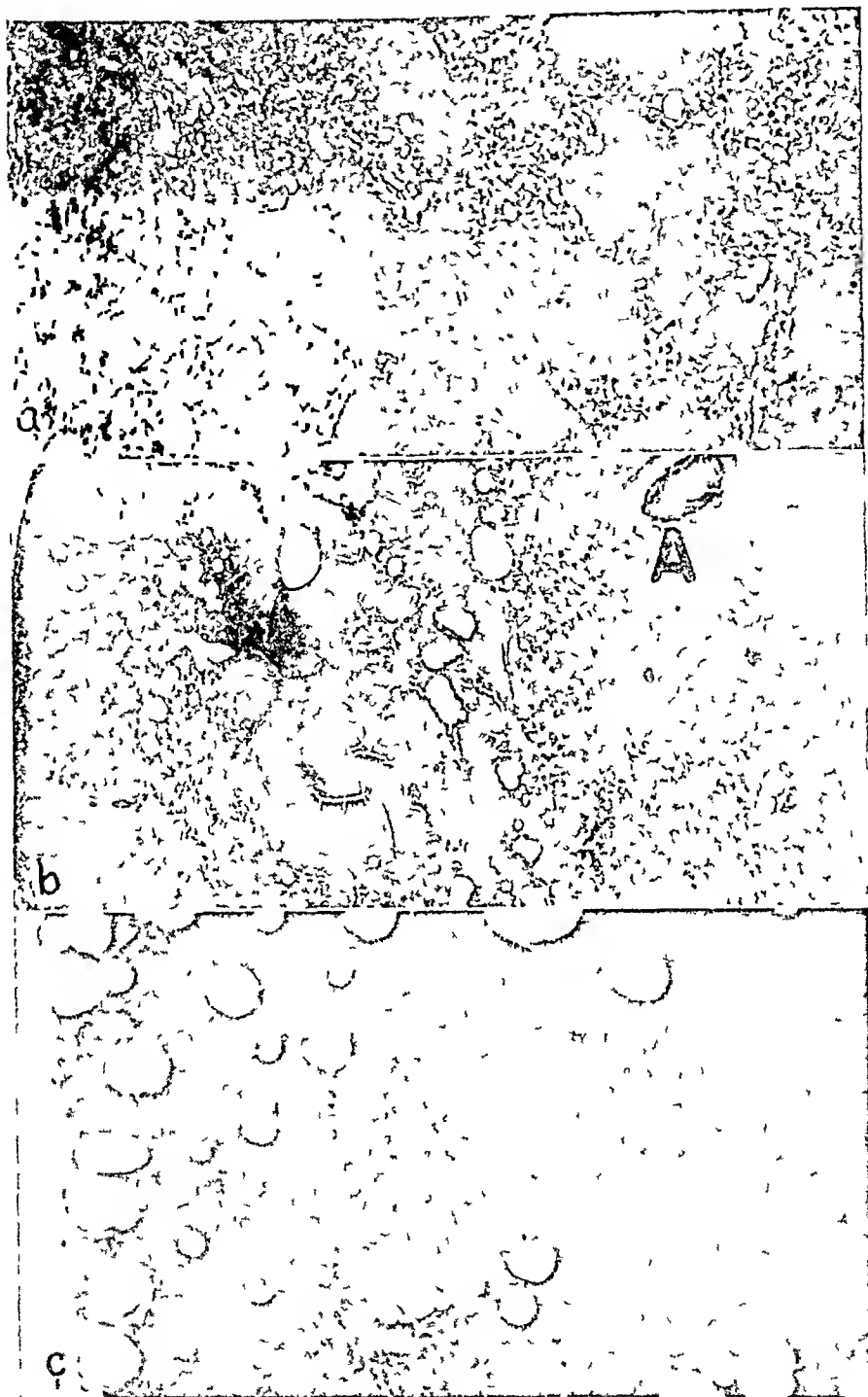


Fig 5—(a) Intense intrapulmonary hemorrhage involving the pleura and the subjacent parenchyma $\times 100$ (Army Institute of Pathology negative 90908) (b) Intense intrapulmonary hemorrhage involving the pleura and a deeply seated parenchymal focus. The intervening lung tissue is relatively free of hemorrhage. The thrombus in the large pulmonary vessel at *A* occurred post mortem $\times 6$ (Army Institute of Pathology negative 90907) (c) Intense intrapulmonary hemorrhage involving the lung parenchyma but sparing the pleura. Small air vesicles are lined up beneath the pleura $\times 35$ (Army Institute of Pathology negative 90900)

In all three photomicrographs the pleura is on the left

(f) Mesentery and Omentum A massive hemorrhage measuring 7 by 9 cm and several smaller hemorrhages were found in the mesentery in 1 case in which there were no hemorrhages or perforations in the stomach or the intestines. In another, small hemorrhages were seen in the omentum.

(g) Gastrointestinal Tract In 1 case a very extensive hemorrhage of the major portion of the body and cardia of the stomach involved all the layers of the wall, but was especially pronounced in the submucosa and the mucosa. Associated with it were submucosal edema and thrombosis of many of the sub-

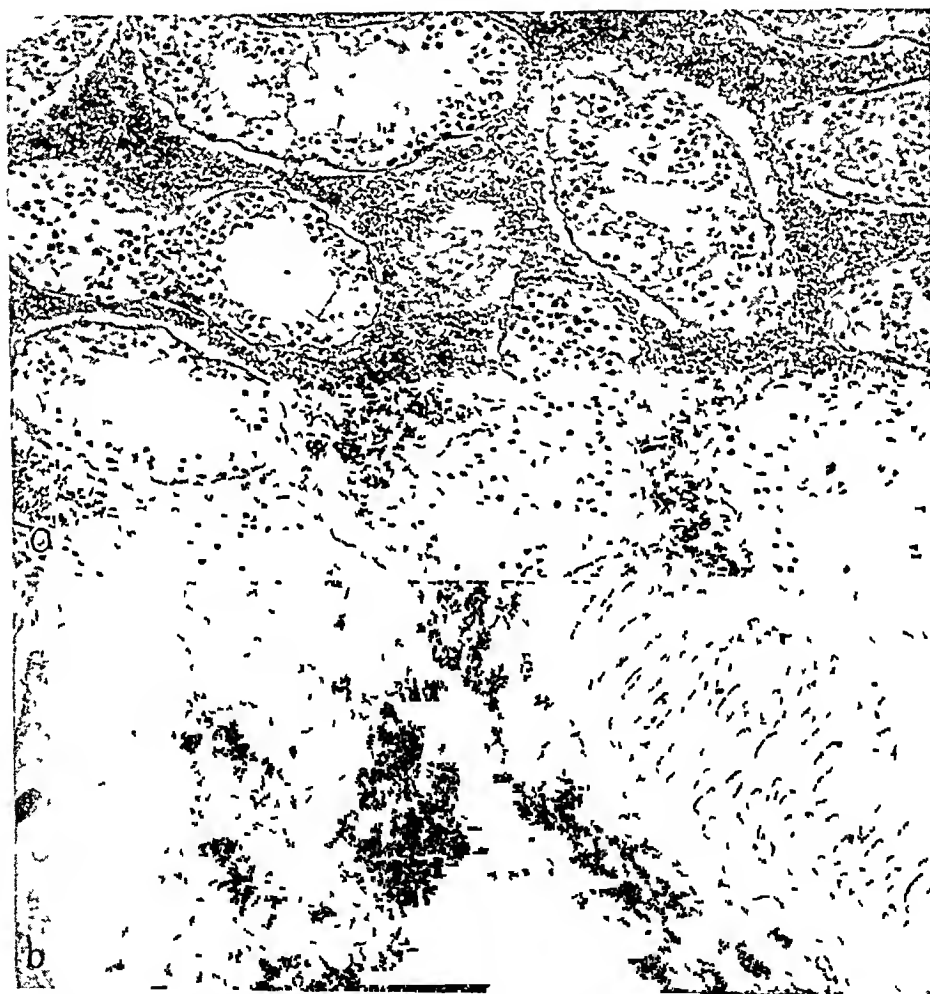


Fig 6—(a) Testis showing intense hemorrhage in the interstitial tissue $\times 116$ (Army Institute of Pathology negative 90896) (b) Sigmoid showing intense hemorrhage in the submucosa extending into the inner muscularis $\times 145$ (Army Institute of Pathology negative 90904)

mucosal vessels, with polymorphonuclear leukocytes infiltrating the area heavily, especially the submucosa and the mucosa. In another there was an acute 2 cm perforation of the sigmoid opposite the mesenteric attachment, 25 cm proximal to the anal orifice. In the vicinity of this "blow-out" were extensive hemorrhages especially in the submucosa (fig 6 b), with polymorphonuclear leukocytes heavily infiltrating the entire thickness of the wall of the sigmoid, as well as diffuse fibrinopurulent peritonitis. In a third case the wall of the colon contained patchy, small hem-

orrhages They were located in the submucosa, the subserosa and the adjacent mesentery

(h) Brain Pronounced hyperemia of the leptomeninges and the brain substance was a constant finding Diffuse leptomeningeal hemorrhages, present in 4 cases, were severe in 1 and moderate in 3 (fig 7 a) Intracerebral hemorrhages were noted in 5 cases In 1 case there were an extensive hemorrhage and a

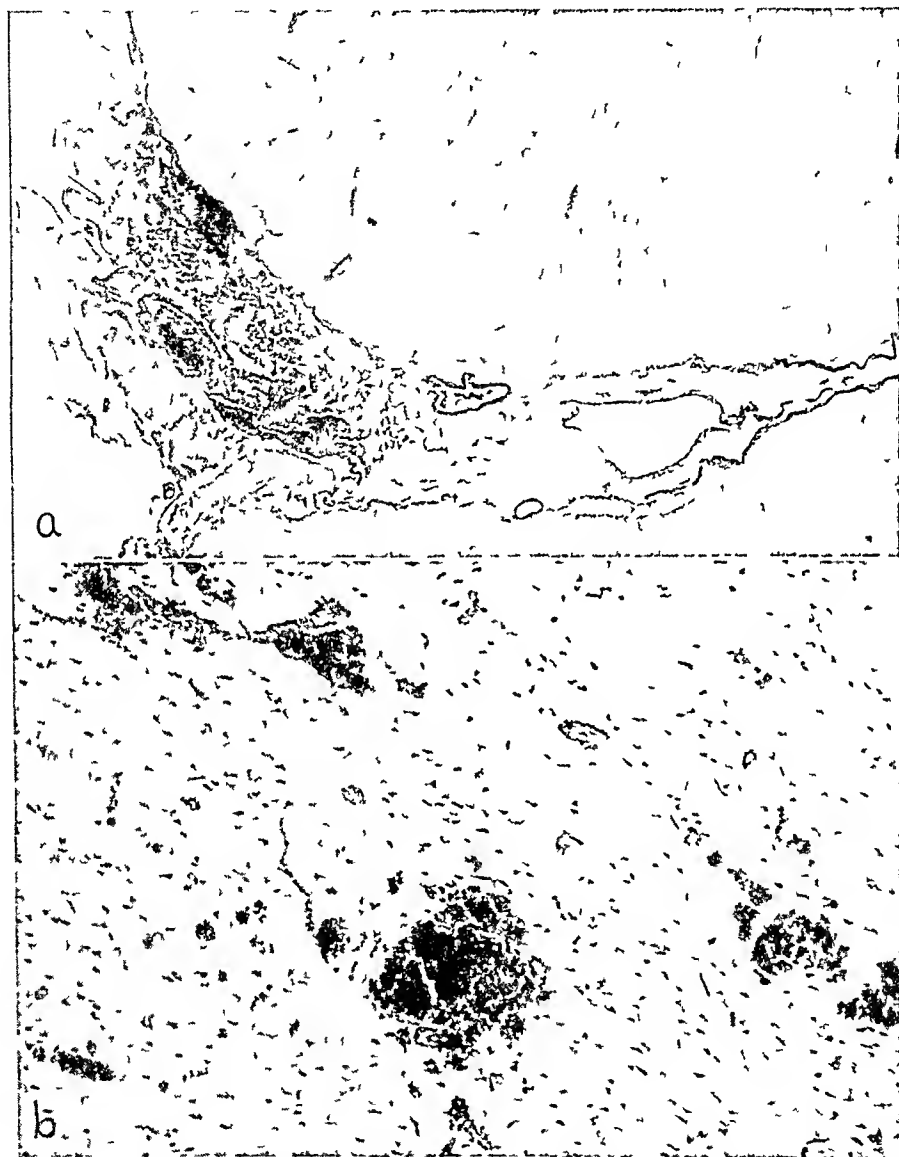


Fig 7—(a) Focal hemorrhage of the leptomeninges of the cerebrum $\times 27$ (Army Institute of Pathology negative 90898) (b) Punctate hemorrhages of the cortex of the temporal lobe $\times 54$ (Army Institute of Pathology negative 90897)

horizontal laceration in the vermis and both hemispheres of the cerebellum and, in addition, many small punctate hemorrhages in the cerebrum In another the hemorrhages consisted of multiple, discrete, red, punctiform spots involving 1 to 2 cm of the cortex of the right temporal lobe (fig 7 b) In the other 3 cases

the intracerebral hemorrhages were seen only microscopically. They were few and were usually located in the perivascular spaces. In 3 cases the intracerebral hemorrhage was associated with bleeding into the leptomeninges.

(i) Tympanic Membranes. The tympanic membranes were examined in only 1 case, both membranes were perforated.

(j) Eyes. Bulbar conjunctival hemorrhages were noted in 2 cases, and unilateral intraocular hemorrhage in 1 case.

(k) Miscellaneous. A large hemorrhage occurred in the thymus and perithymic tissues in 2 cases. In 2 cases there were small hemorrhages in the parietal pericardium. Hemorrhage of the diaphragm near the esophageal hiatus occurred once, mediastinal emphysema twice and subcutaneous emphysema of the chest once.

Gross or microscopic tears were never found in the blood vessels of the organs and tissues in which there were hemorrhages.

(l) Liver, Gallbladder, Urinary Bladder, Pancreas, Lymph Nodes, Thyroid Gland and Pituitary Gland. All these lacked lesions which could be attributed to blast injuries.

A summary of the clinical data and postmortem findings is given in table 1.

COMMENT

The term "blast injury" refers specifically to the consequences of the destructive force of the pressure wave set up by the detonation of high explosives. If air is the medium in which the explosion occurs, the term "air blast" or "atmospheric blast" is employed, if water is the medium, "immersion blast" or "underwater blast". The lesions in pure blast injuries are almost exclusively visceral. The bones and the soft tissues of the body wall and of the extremities usually are not involved.

The fundamental experimental studies on the nature, the mode of action and the effects of blast were performed by Hooker¹ and by Zuckerman and associates² for air blast and by Williams,³ Cameron, Short and Wakely,⁴ Greaves and associates,⁵ Friedell and Ecklund⁶ and Friedell and Burke⁷ for immersion blast. Zuckerman's comprehensive studies have laid the groundwork for present day knowledge of blast injuries. Physically the blast wave has two components, a positive or

1 Hooker, D. R. *Am J Physiol* **67** 219, 1924.

2 (a) Zuckerman, S. *Lancet* **2** 219, 1940. (b) Zuckerman, S., Hadfield, G., O'Reilly, J. N., Alston, J. M., and Barsby, B. *Proc Roy Soc Med* **34**:171, 1941. (c) Krohn, P. L., Whitteridge, D., and Zuckerman, S. *Lancet* **1** 252, 1942.

3 Williams, E. R. P. *Brit J Surg* **30** 38, 1942.

4 Cameron, G. R., Short, R. H. D., and Wakeley, C. P. G. *Brit J Surg* **30** 49, 1942.

5 Greaves, F. C., Draeger, R. H., Brines, O. A., Shaver, J. S., and Corey, E. L. *U S Nav M Bull* **41** 339, 1943.

6 Friedell, M. T., and Ecklund, A. M. *U S Nav M Bull* **41** 353, 1943.

7 Friedell, M. T., and Burke, R. *U S Nav M Bull* **41** 363, 1943.

compression factor and a negative or suction factor⁸ The positive wave attains greater intensity, reaches its peak more abruptly and persists for a shorter time than the negative wave The duration of each component is only a small fraction of a second^{2a} Because air is less dense and thus more readily compressed than water, water-borne pressure waves reach their maximal intensity later and subside more slowly than air-borne pressure waves Hence the effects of a given charge of detonated high explosive will be felt over a much greater distance in water than in air In water to all intents and purposes there is no suction component³ Therein lie the essential differences between underwater and atmospheric blast waves As would be expected, the mode of action and the effects on the body of the two forms of blast are basically similar Underwater explosions, however, produce intra-abdominal lesions relatively more often than explosions in air, but the characteristic and almost constant lesion in both forms of blast occurs in the lungs

Critical experiments have demonstrated rather conclusively that the pulmonary lesions of air blast are due to the direct impact on the body wall of the pressure component of the blast wave, not to a sudden rushing of air under pressure down the respiratory tree and into the lungs The genesis of the gastrointestinal lesions is similar, the intestinal injuries cannot be attributed to a sudden forcing of air or fluid (in the case of immersion blast) into either end of the gastrointestinal tract The evidence does not support the belief that in air blast the pulmonary lesions result from a lowering of the intrapulmonary pressure by the suction component of the blast wave, acting through the respiratory passages²¹ The impact of the positive pressure wave on the thoracic wall is primarily responsible for the pulmonary damage, but it is conceivable that the suction wave which follows might add to the insult by pulling out the wall of the chest, thus stretching the already injured lung tissue^{2b}

Perhaps the most characteristic feature of blast injuries is the vulnerability of the air-containing organs and the relative immunity of the solid viscera to the damaging effects of the blast wave This difference in visceral response to blast is best explained by certain well established physical principles When a transmitted high pressure wave passes from a medium of greater density (fluid) to one of lesser density (air) a shredding or disruptive effect occurs at the interface of the media⁹ We may consider the soft tissues and the solid parenchymatous organs as representing a fluid medium, and the lungs and the intestine (if gas

8 Sutherland, G A Lancet 2 641, 1940 Hooker¹ Zuckerman^{2a}

9 Clark, S L Quart Bull Northwestern Univ M School 18 81, 1944 Williams³ Greaves and others⁵

is present in the lumen) a gaseous medium. The blast wave as it passes through the skin, the subcutaneous tissues, the muscles, the liver, the spleen, the kidneys and other structures will produce relatively little damage. But when the pressure wave breaks out of the liquid medium into the gaseous medium (lungs, intestine), the bursting or shredding effect is great enough to produce serious disruption of tissue, resulting in hemorrhage and at times perforation. Ordinarily the stomach and the intestines are resistant, but if gas is present in their lumens the wave will shred as it breaks through.⁵ If the gas can be displaced without compression, a hemorrhagic lesion of the wall will be the only result.⁵ Perforation will occur when the gas bubble is trapped, becomes compressed under the pressure of the wave and snaps back in reexpansion when the wave passes.⁵ If the wall is sufficiently weakened by the shredding injury, the reexpanding bubble will produce a "blow-out" perforation.⁵

A blast wave passing through the body behaves according to a fairly definite pattern, and in every instance it produces constant lesions in man as well as in various experimental animals. The constant and most significant lesion is in the lungs, mainly in the form of extensive bilateral traumatic hemorrhages. Generally the severity of the pulmonary hemorrhages varies directly with the size of the exploding charge and inversely with the distance of the body from the explosion. Hooker¹ regularly found bilateral intrapulmonary hemorrhages in frogs, rabbits, cats and dogs exposed to gun blast. Zuckerman^{2a} observed similar lesions after subjecting mice, rats, guinea pigs, rabbits, cats, monkeys and pigeons to detonated charges of high explosive or of hydrogen and oxygen contained in balloons. In man, pulmonary hemorrhages were always found in the cases of blast reported by Falla,¹⁰ Hadfield and co-workers,¹¹ Hadfield and Christie,¹² O'Reilly and Gloyne¹³ and Wilson and Tunbridge.^{13a} In our series bilateral hemorrhages of the lungs occurred in all the cases. Zuckerman and co-workers^{2c} found that the hemorrhages of the lungs were immediate in onset, as they were observed within one minute after exposure to the blast. Hadfield,^{2b} on the contrary, expressed the belief that the maximal amount of intrapulmonary bleeding did not occur immediately, but that the hemorrhages continued, reaching their maximum as much as fifty-one hours later. His opinion was based on the observation that the hemorrhages were smaller when death occurred instantaneously than when there was a period of survival. The

10 Falla, S. T. *Brit M J* **2** 255, 1940

11 Hadfield, G., Ross, J. M., Swain, R. H. A., Drury-White, J. M., and Jordan, A. *Lancet* **2** 478, 1940

12 Hadfield, G., and Christie, R. V. *Brit M J* **1** 77, 1941

13 O'Reilly, J. N., and Gloyne, S. R. *Lancet* **2** 423, 1941

13a Wilson, J. V., and Tunbridge, R. E. *Lancet* **1** 257, 1943

findings in our cases indicate that the maximal pulmonary damage occurs very early, for the survival time in cases 3 and 7 was less than one hour and yet the hemorrhages were as marked as in any of the other cases, and the hemorrhages in case 6 were the least severe though the survival time was seventy-one hours. That the hemorrhages may not be altogether due to tears or ruptures of alveolar walls seems probable, for in many areas of hemorrhage histologically intact septums were noted. Both Hadfield^{2b} and Cameron, Short and Wakely⁴ shared this view. They expressed the belief that the ruptured capillaries were far too few to account for such extensive hemorrhage. They attributed most of the bleeding to diapedesis of red cells. Zuckerman^{2b} adhered to the opinion that the bleeding was due primarily to rupture or tearing of the alveolar walls. We failed to find the intense generalized dilation and congestion of septal capillaries reported by Hadfield^{2b}, on the contrary, we often observed compressed and empty capillaries in the intensely hemorrhagic patches.

Acute pulmonary emphysema was seen frequently and was almost always of the vesicular variety. Presumably it was due principally to the dilation of alveoli, alveolar ducts and respiratory bronchioles when air became trapped or displaced by a combination of the action of the blast wave and the outpouring of blood. It resulted also from the coalescence of adjacent air spaces when the intervening septums were torn. In only 2 cases did we observe air in the interstitial tissue of the lung. The emphysema was apparent grossly in 1 of our cases. From the results of a series of experiments in which animals were exposed to explosion of underwater depth charges, Cameron, Short and Wakeley⁴ were able to correlate the degree of pulmonary damage with the type of emphysema. They found vesicular emphysema in the majority of their animals (regardless of distance from the charge), and interstitial emphysema only in those close to the explosion. In general the animals in the latter group showed the more serious pulmonary damage. The infrequency of interstitial emphysema in our cases suggests that the injury of the lungs must be of the most severe grade before air is driven into the interstitial tissues.

Lesions of the tracheobronchial walls (intramural hemorrhages and occasional epithelial desquamation) are mentioned in the literature on blast injuries¹⁴. Neither gross nor microscopic lesions were observed in the walls of the trachea and the bronchi in our cases, but the bronchioles shared in the injury of the remainder of the lung, and intramural lesions were repeatedly observed in them. Many bronchioles showed nothing more than loss of epithelium, with or without loss of lamina propria, and minimal to moderate leukocytic infiltration of

14 Zuckerman and others^{2b} Cameron and others⁴ Falla¹⁰ O'Reilly and Gloyne¹³

the remaining lamina propria and occasionally of the muscle. The absence of leukocytes in the bronchiolar lumen and of significant inflammatory reaction in the surrounding pulmonary parenchyma suggested that the intramural leukocytic reaction was in response to an intrinsic injury of the bronchioles.

The separation of the epithelium that was often observed in the bronchiolar wall (fig. 3 and 4) is not to be confused with that commonly seen in postmortem material, a phenomenon which pathologists generally regard as an artefact. Such postmortem change differs from the type of separation we have described, since in the former there is no associated hemorrhage, subepithelial accumulation of edema fluid or leukocytic infiltration of the wall. The situation of the bronchiolar epithelium increases its susceptibility to blast injury, because it lies precisely at the fluid-air interface, where the disruptive effect of the pressure wave would strike. There is little room for doubt that blast can cause great damage to the bronchioles.

Examinations have been made for fat embolism in blast injuries by many investigators on material from both experimental animals and man. The results have been repeatedly negative¹⁵ except in the case of an air raid casualty reported by Robb-Smith¹⁶ in which fat embolism was severe and was considered a major cause of death. In all 11 cases of our series sections of the lung were stained (sudan III) for fat. Fat embolism was present in 8, the amount of fat was moderate in 3 and minimal in 5. In the 3 cases in which pulmonary fat embolism was moderate, there were superficial burns. Since fat embolism is known to occur following burns,¹⁷ it is impossible to decide whether in our cases the burns or the blast injuries were responsible for the dissemination of fat. Regardless of the causative agent, we may be reasonably certain that the amount of fat was not great enough in any of our cases to have significantly affected the clinical course or to have been materially responsible for the lesions observed in the lungs. Reports in the literature and our experience justify the conclusion that fat embolism is not an important consideration in the majority of cases of blast injury.

Pneumonia complicating blast injury of the lungs of experimental animals and man has been reported¹⁸. The pneumonia may develop within a matter of hours after the blast, and the infection may alter the clinical course. In most of our cases there was minimal to moder-

15 (a) Wilson, J. V., and Tunbridge, R. E. *Lancet* **1** 257, 1943. (b) McKibben, P. S. *Am J Physiol* **48** 331, 1919. (c) Zuckerman and others^{2b}. (d) Cameron and others⁴. (e) O'Reilly and Gloyne¹³.

16 Robb-Smith, A. H. T. *Lancet* **1** 135, 1941.

17 Groskloss, H. H. *Yale J Biol & Med* **8** 175, 1935.

18 Zuckerman and others^{2b}. O'Reilly and Gloyne¹³.

ate exudation of leukocytes and large mononuclear cells in the hemorrhagic as well as in the nonhemorrhagic portions of the lung. However, in only 4 of the cases was the inflammatory response sufficiently heavy to warrant the diagnosis of bronchopneumonia. Notwithstanding the presence of pneumonia, we believe that death was due primarily to the extensive pulmonary damage incident to the blast injury. It is interesting that in 2 of the 4 cases pneumonia developed despite early and continued administration of large doses of penicillin. Conditions under which the autopsies were performed were such that samples from the lungs could not be obtained for bacteriologic study.

Since in 6 of our cases superficial burns were fairly extensive and since burns are known to produce changes in the lungs, the question whether the burns were causally related to the pulmonary lesions must be considered. In burns the principal pulmonary findings are hyperemia, edema and petechiae.¹⁹ Massive pulmonary hemorrhages and severe emphysema are not described, nor have we ever encountered them at autopsy. Furthermore, in the 5 cases without burns the pulmonary lesions were the same as in those with burns, and the lesions were just as pronounced when only a small area of the body was burned as when as much as 40 per cent was involved. We believe, therefore, that the burns were of little or no significance in the causation of the pulmonary lesions.

We may dismiss inhalation of irritant or toxic gases as a possible etiologic factor in the pulmonary lesions in this series. The explosions occurred out of doors in the open air, and the periods of exposure were brief. Furthermore, the hemorrhagic and necrotizing inflammation of the trachea and bronchi seen following inhalation of irritant fumes²⁰ was uniformly absent.

Carbon monoxide intoxication also may be eliminated because the exposure was in the open air and brief, and the typical cherry red coloration of the blood, the viscera and the muscles was absent. Carboxyhemoglobin saturation of the blood was not determined.

In addition to its effects on the lungs, blast may injure other organs and tissues. In the literature there is reference to involvement of most of the solid parenchymatous organs of the abdomen,²¹ the urinary bladder,^{2b} the pregnant uterus,^{2b} the gastrointestinal tract,²² the mesentery,^{15a} the omentum,³ the retroperitoneal tissues,²³

19 Pack, G. T. *Arch Path* **1** 767, 1926. Erb, I. H., Morgan, E. M., and Farmer, A. W. *Ann Surg* **117** 234, 1943.

20 Mallory, T. B., and Brickley, W. J. *Ann Surg* **117** 865, 1943.

21 Zuckerman and others^{2b} Cameron and others⁴ O'Reilly and Gloyne¹³

22 (a) Ecklund, A. M. *U. S. Nav. M. Bull.* **41** 19, 1943. (b) Zuckerman and others^{2b} (c) Williams³ (d) Cameron and others⁴ (e) Greaves and others⁵ (f) Friedell and Ecklund⁶ (g) O'Reilly and Gloyne¹³

23 Williams³ O'Reilly and Gloyne¹³

the anterior and posterior mediastinal tissues,²⁴ the thymus,^{2b} the heart,²⁵ the aorta (rupture of it),^{15a} the soft tissues of the chest and the abdomen,²⁶ the soft tissues of the neck,²⁴ the periorbital soft tissues,^{2b} the eyes,³ the tympanic membranes²⁷ and the central nervous system²⁸ There also have been reported hemoperitoneum,²⁹ hemothorax³⁰ and hemopericardium³¹ The extrapulmonary lesions almost always were focal hemorrhages in the organs or tissues affected, lacerations were noted but were rare, occasionally perforations occurred in the intestine, in the majority of cases the tympanic membranes were perforated The hemorrhagic lesions were usually small, but occasionally in the soft tissues of the thoracic and abdominal walls, the retroperitoneal tissues and the anterior and posterior mediastinal tissues the hemorrhages were of huge dimensions In our series the locations of the hemorrhages are summarized in table 1 It is plain that they were widely distributed Particular reference should be made to the testicular hemorrhage seen in case 5 No such observation, as far as we have been able to ascertain, has been reported previously in cases of air or immersion blast The extensive necrosis and venous thrombosis in the spleen in case 10 may possibly be attributed to the burns rather than to the blast Characteristically in blast injuries there are no significant external wounds and the bones are not fractured unless forcible contact occurs between the victim and some object or hard surface

We wish to emphasize that in blast injuries (1) the extrapulmonary lesions are inconstant and are usually inconsequential in the ultimate outcome, (2) the pulmonary hemorrhages are constant and account for the characteristic clinical and pathologic features of blast injuries and (3) in the average case the extent and the severity of the pulmonary hemorrhages are the deciding factors in the survival or the death of the patient

The lesions of the lungs provide the basis for the principal clinical manifestations of blast The severe pulmonary hemorrhages and edema and the overflowing of blood and edema fluid into the respira-

24 Zuckerman and others^{2b} O'Reilly and Gloyne¹³

25 Stewart, O W, Russel, C K, and Cone, W V Lancet **1** 172, 1941
Cameron and others⁴ Wilson and Tunbridge^{15a}

26 Zuckerman and others^{2b} O'Reilly and Gloyne¹³ Wilson and Tunbridge^{15a} Ecklund^{22a}

27 Hooker¹ Zuckerman and others^{2b} Williams³ O'Reilly and Gloyne¹³

28 (a) Mott, F W J Roy Army M Corps **29** 662, 1917 (b) Zuckerman and others^{2b} (c) O'Reilly and Gloyne¹³ (d) Stewart and others²⁵

29 Cameron and others⁴ Wilson and Tunbridge^{15a} Ecklund^{22a}

30 Cameron and others⁴ Greaves and others⁵ O'Reilly and Gloyne¹³
Wilson and Tunbridge^{15a} Ecklund^{22a}

31 Zuckerman and others^{2b} Wilson and Tunbridge^{15a}

tory passages are the causes of the respiratory distress. The respiratory rate is usually increased, this appears to be due at first to massive stimulation of the deflation endings in the lung (the compression effect on lungs as the blast wave strikes the chest)^{2c} Subsequently, after intense hemorrhage and deformation of lung tissue have occurred, the increase in rate apparently results from the increased sensitivity of the intrapulmonary stretch endings.^{2c} The hyperpnea cannot be attributed to chemical changes affecting the respiratory center or to the direct effect of the impact of the blast wave on the medulla.^{2c} The early experiments of Hooker¹ indicated that shock, which so regularly occurs in blast, is not related primarily either to

TABLE 2—*Summary of Symptoms Referable to the Central Nervous System and Pathologic Findings in the Brain in Nine of Eleven Cases of Atmospheric Blast Injuries*

| Case | Symptoms and Signs | Findings in Brain |
|------|--|--|
| 1 | Pronounced restlessness | Hyperemia of leptomeninges and brain, no hemorrhages |
| 2 | Patient excitable, irrational, maniacal | No lesions |
| 3 | Patient dead on arrival at hospital | Diffuse moderate leptomeningeal hemorrhage hyperemia but no hemorrhages of brain |
| 4 | No symptoms or signs mentioned in clinical abstract | Diffuse moderate leptomeningeal hemorrhage focal, punctate, grossly visible hemorrhages in cortex of right temporal lobe |
| 5 | No symptoms or signs mentioned in clinical abstract | Hyperemia of leptomeninges and brain occasional microscopic perivascular hemorrhages in brain |
| 6 | Stupor followed by extreme restlessness and irritability | Hyperemia of leptomeninges and brain focal microscopic leptomeningeal and intracerebral hemorrhages |
| 8 | No symptoms or signs mentioned in clinical abstract | Hyperemia of leptomeninges and brain |
| 9 | Patient unconscious, disoriented, excitable, hyperactive reflexes, bradypnea | Large horizontal tear and massive hemorrhage in cerebellum punctate hemorrhages in cerebrum diffuse severe leptomeningeal hemorrhage |
| 11 | No symptoms or signs mentioned in clinical abstract | Hyperemia of leptomeninges and brain, occasional microscopic perivascular hemorrhage in brain |

the peak blast pressure or to the presence of the intrapulmonary hemorrhages. He stressed, as the causative factor the duration of the positive pressure component of the blast wave. The more recent studies of Krohn and co-workers^{2c} suggest that the contrary is true—namely, that the extent and the duration of the fall in arterial blood pressure are related to the peak blast pressure experienced and to the changes in the pulmonary capillary bed incident to the hemorrhages in the lungs. The extremely slow respiratory rate noted in case 9 in all likelihood was due to the severe damage of the brain. Blast effect on the lungs may be readily detected roentgenologically, since the intrapulmonary hemorrhages cast characteristic shadows.

Symptoms referable to the central nervous system were commonly observed in our series. In table 2 these symptoms and the findings

in the brain are summarized for the 9 cases in which the brains were examined. Special cytologic studies of the nerve cells were not done. In our series it appears that no consistent correlation can be made between symptoms and pathologic findings. Under combat conditions, when casualties are being received in large numbers, detailed clinical observations are not possible. This may apply to case 4, in which despite diffuse leptomeningeal hemorrhages and focal punctate intracerebral hemorrhages, no symptoms referable to the central nervous system were noted. In other cases active symptoms of involvement of the central nervous system are recorded in the absence of brain or meningeal hemorrhages (case 2). In the series of cases of blast injury reported by O'Reilly and Gloyne¹³ restlessness, extreme in some instances, was an almost constant symptom, "some subarachnoid hemorrhage" was a postmortem finding in 2. In a case reported by Mott^{28a} mania was a prominent symptom. The brain showed scattered subpial and intracerebral hemorrhages, diffuse venous congestion and early chromatolytic changes in the nerve cells. Mott expressed the belief that the vascular lesions were responsible for the acute excitability manifested by the patient. Krohn and co-workers^{2c} observed that animals exposed to blast exhibited normal cortical activity for ten to twenty seconds after the explosion and then a subsequent depression (but not a cessation) of activity. In animals struck by a blow on the head the reaction was different, here cortical activity was immediately abolished and reappeared after about three minutes. Their observations also indicated that concussion had not occurred. Zuckerman and co-workers^{2b} repeatedly found that blast pressure sufficient to cause death and extensive pulmonary hemorrhages failed to produce any lesions in the brain. In subsequent experiments, in which much higher pressures were employed (many times the lethal level) some of the animals showed hemorrhage and edema in the spinal cord and subpial and intraventricular hemorrhage in the brain, but in the brain substance hemorrhage was not found. It appears evident, then, that blast may produce hemorrhages and certain functional alterations in the brain. The specific role which these play in the causation of the symptoms and the other factors that may be operating cannot be accurately assessed.

SUMMARY AND CONCLUSIONS

The principal and characteristic lesion in 11 selected cases of atmospheric blast injuries was severe diffuse bilateral traumatic hemorrhage of the lungs. All lobes of both lungs and all portions of a given lobe were involved in the hemorrhagic process. Intense edema and emphysema of the lungs occurred almost as frequently as did hemorrhage. The emphysema was predominantly vesicular in type,

the interstitial variety was noted only twice. The bronchioles, though perhaps not so vulnerable as the remainder of the pulmonary parenchyma, also were susceptible to the damaging effects of the blast wave, the epithelium became loosened or detached from the lamina propria, with erythrocytes, edema fluid and leukocytes being extravasated into the injured bronchiolar wall. Pulmonary fat embolism was moderate in 3 and minimal in 5 cases. We regard this degree of fat embolism as neither of significance in the causation of the pulmonary lesions nor of influence in the clinical course of blast injury.

Although the predominant damage of blast is intrapulmonary, none of the soft tissues or other viscera shows absolute immunity to the destructive effects of the blast wave. In every case extrapulmonary lesions occurred in a variety of organs as small hemorrhages or very occasionally as tiny lacerations in the affected structures. In 4 cases striking extrapulmonary lesions were found. In 1 case there was a "blow-out" perforation of the sigmoid, in a second, a massive hemorrhage in the mesentery, in a third, a large horizontal tear and a massive hemorrhage in the cerebellum, in a fourth an extensive hemorrhage in the wall of the stomach.

Six patients were burned. Though it is recognized that changes in the lungs frequently occur with burns, we are convinced that the burns were not responsible for the pulmonary lesions in this series of cases. It is possible that they were instrumental in causing the pulmonary fat embolism observed in 3 cases and the necrosis of the spleen in 1 case. Bronchopneumonia occurred in 4 cases. Hemothorax was noted in 7 cases, mediastinal emphysema in 2, pneumothorax in 1 and subcutaneous emphysema in 1.

Our findings in general are in accord with the observations of other investigators who have studied atmospheric blast injuries in animals and man.

STRUCTURE OF THE TESTIS IN INFANCY AND IN CHILDHOOD

With a Discussion of the So-Called Underdeveloped Testis

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THE MINUTE structure of the immature testis has been the subject of a number of investigations reported in the literature. The majority of these previous communications dealt with the problem of defining the normal condition of the testis as observed in infancy and childhood, in order to provide a basis on which the condition of a particular organ may be evaluated. Several early workers in this field brought an unfortunate trend into the investigation of the immature testis by designating the great majority of the organs at their disposal as abnormal. Much of the following work has been concerned with arguments about this point. These and other reports in the literature will be discussed more fully in a later section of this report.

The presence of large numbers of well preserved, presumably normal testes in the material of this laboratory suggested a new examination of the structure of the immature testis. An effort was made to abstain from preconceived criteria of normalcy and to designate as normal that range of forms which must be assumed to develop at puberty into structurally and functionally normal testes.

MATERIAL AND METHODS

All organs used in the present investigation were obtained at autopsies and fixed in Zenker's fluid. The histologic condition of the tissue, rather than the time elapsed between death and fixation, was taken into consideration in selecting the specimens, since it is known that the speed of postmortem deterioration of tissues varies greatly. Sections were thus selected from well over 100 suitable testes and stained with hematoxylin and eosin. Only 1 organ was examined in each case. In 97 cases various special stains and staining methods were employed, such as phloxine and methylene blue, Mallory's connective tissue stain, phosphotungstic acid-hematoxylin, or Foot's or Gomori's silver impregnation of lattice fibers. No use was made of various histochemical or physical methods for the demonstration of substances related to the specific function of endocrine cells,¹ because it was the aim of the study to describe those structural characteristics

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1 Dempsey, E W, and Bassett, D L. *Endocrinology* **33** 384, 1943

which may be demonstrated by the methods available in the average histologic or pathologic laboratory

The ages of the persons whose testes were studied for the present report ranged from birth to 12 years, including a number of premature infants. The changes at puberty are therefore not included. The age distribution of the specimens studied with special stains (that is, other than hematoxylin and eosin) was as follows: first day, 8, 1 to 7 days, 15, 7 days to 1 month, 14, 1 to 3 months, 15, 3 to 6 months, 13, 6 to 12 months, 11, 1 to 4 years, 11, 4 to 12 years, 10. Testes which were obviously affected by disease were excluded. Almost all organs selected according to their state of preservation were within the limits of what is here considered to be normal. The history and the cause of death were not evaluated in detail in each case. Only in a few instances the question of a developmental disturbance arose, and in those the history was carefully studied.

OBSERVATIONS

The developmental changes will now be described separately for each histologic constituent of the testis. This facilitates the description and involves no considerable disadvantage since there is no obvious correlation of changes in various tissues of the testis during the period under consideration.

Sex Cords and Seminiferous Tubules.—The presence of large numbers of hollow seminiferous tubules is uncommon before puberty. The great majority of the organs contain solid sex cords throughout. Embryologically these are primary sex cords, that is, derived from the first generation of cords forming in both sexes during the early phases of gonadal development. The occurrence of the secondary sex cords of the testis will be discussed in the following section.

Several steps of development precede the formation of lumens in the sex cords of the testis. The first of these, recognizable even before birth, consists in a characteristic radial arrangement of the oblong nuclei of the future Sertoli cells within the cords. These nuclei are more densely arranged in the peripheral parts of the cords. A number of the testes of the first year showed this condition, although the majority exhibited the next stage, in which the nuclei are present only peripherally in the cords, leaving a central portion free (figs 1, 4 and 7). This central area is filled with pale and irregularly staining material forming granules or meshes. It is impossible to determine whether this mass consists of parts of the cell bodies completely or partly filling the central area, or of debris of parts of cells, or of entire cells disintegrating in the center of the cord, or of coagulated extracellular material. There is, however, no free lumen present in this stage of differentiation. Only a small number of specimens showed patent seminiferous tubules, and even in these organs many of the cords were still in the stage just described. When definite lumens were present cells were often seen in them, with large nuclei and cell bodies of irregular form (fig 5), and one might suspect that they remained behind when the other cells retracted to the periphery. There is no indication that the frequent occurrence of these cells in seminiferous tubules of children is in any way abnormal. Similar cells may also be seen in the centers of cords of the preceding stage.

These changes in the sex cords do not occur simultaneously in all parts of a testis. Thus two or sometimes all three of the stages may be seen in adjacent cords. Their distribution, as far as age is concerned, is so irregular that it could be evaluated statistically only in a material much larger than that at our disposal. In judging single testes, these changes are therefore of minor importance. The

uniformly solid cords, as described in a foregoing paragraph, occur mainly during the first year. Lumen formation, on the other hand, is seen more commonly in the testes of older children. This is not a rigid rule, and the youngest testis with definite lumens in some of its tubules was that of a 1 day old infant. The great majority of the organs examined showed most of their cords having pale-staining centers, described in the foregoing paragraph as the second stage. However, shrinkage due to inadequate preservation may well produce the semblance of lumens in these cords.

The basal membranes of the sex cords will be described in the section on the connective tissue. The spermatogonia show no significant changes during childhood.

Secondary Sex Cords in Normal Testes—Embryologic studies have shown that secondary sex cords, which were formerly believed to form only in ovaries, also occur in some normal human testes.² These cords were found in groups within the tunica albuginea, or between it and the superficial epithelium with which they connect. They often penetrate the tunica albuginea and join with the typical (primary) cords in the interior of the organ. They occur in embryos of varying ages, and no clear concept could be gained of the frequency with which they occur, since they are often overlooked unless serial sections of the entire testis are prepared. Furthermore, it is not known how long these cords can be distinguished from the primary ones, because all or most of them probably join the primary cords and are eventually covered by a newly formed portion of the tunica albuginea, so that they can no longer be distinguished. Meyer³ described these cords as observed in the testes of 2 fetuses of 7 months, and explained them as being cut off from the rest of the cords by the developing tunica albuginea. More recently Chin⁴ described such cords as seen in 6 subjects, including fetuses, infants and children, and explained them in a similar manner. The present material contains 2 testes, of 1 and 9 days, respectively, with similar groups of secondary sex cords (figs 1 and 2). The aforementioned recent embryologic results allow definitely the explanation of all of these findings as secondary sex cords. It is important to know them as vestigial structures in normal testes, and homologues of normal constituents of the ovaries, rather than adenomatous growths or signs of intersexual development, unless they occur in much larger amounts.⁵ From the mechanical point of view, groups of secondary sex cords may be of interest if they pierce or weaken the tunica albuginea. Considering the soft consistency of the interior of the testis, and the firm texture of the tunica albuginea, one may suspect that any weakening of the latter would predispose the testis to rupture from mechanical causes. Five cases of rupture of a testis reviewed by Counseller and Pratt,⁶ and a case in which a child was recently treated for this condition at this hospital, emphasize the importance of weak points in the tunica albuginea. (In rupture of a testis the tissue at the site of rupture is usually destroyed and can no longer be examined.)

It is impossible at this time to estimate the frequency of secondary sex cords in the testes of infants and children. The areas are small, and even if they were

2 Gruenwald, P. *Am J Anat* **70** 359, 1942

3 Meyer, R. *Ergebn d allg Path u path Anat* **15** 430, 1911

4 Chin, S. *Gann* **31** 694, 1937

5 For a description of large layers of secondary sex cords in testes of intersexes see Gruenwald, P. *Ztschr f Anat u Entwcklungsgesch* **103** 278, 1934
Walther, R. *Beitr z path Anat u z allg Path* **95** 297, 1935

6 Counseller, V S, and Pratt, J H. *J Urol* **52** 334, 1944

present in every testis, the probability of seeing them on single sections cut at random might not be greater than the incidence in the present material

Rete Testis—Not much need be said about the rete testis. Its tubules develop from thin, solid cords, and lumen formation begins much earlier than in the sex cords. It is well under way at birth, and in all stages examined a part of the rete shows definite lumens. There is considerable variation in the proportion of

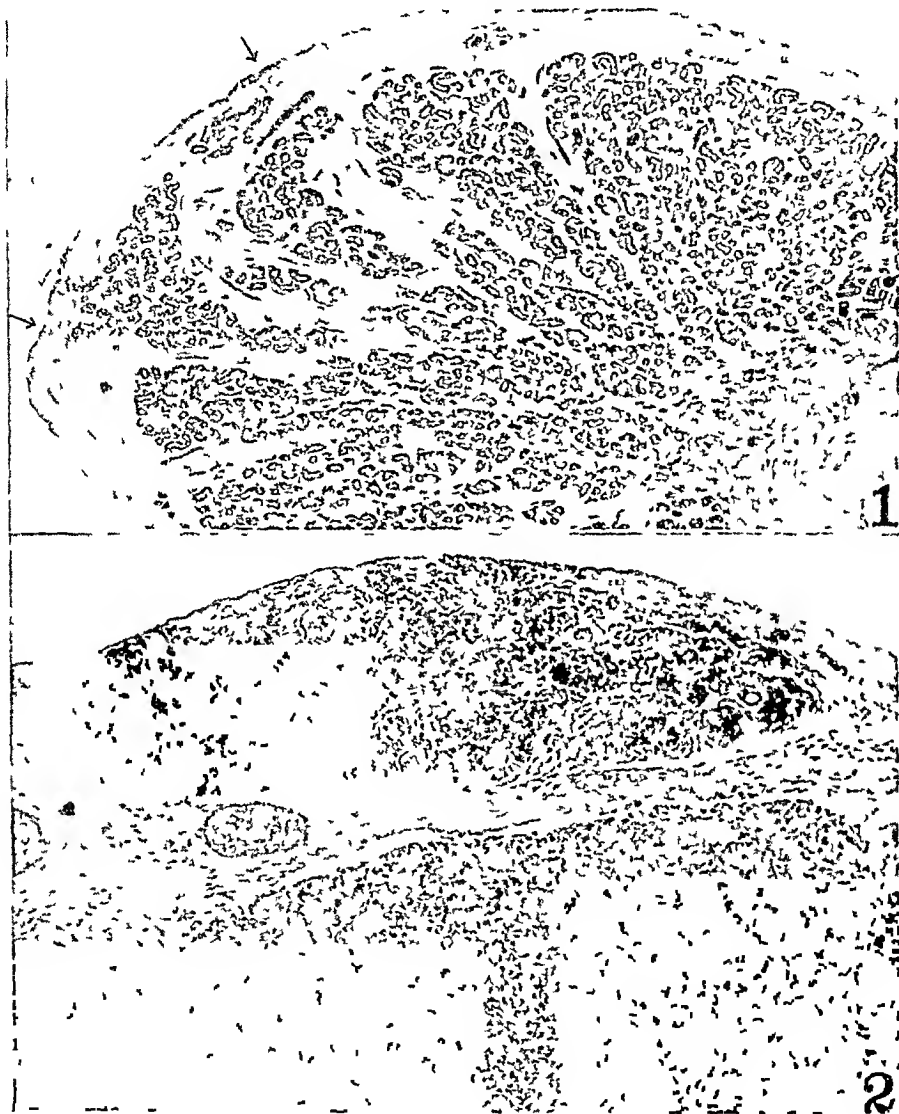


Fig 1—Testis of a 1 day old premature infant. A group of secondary sex cords is seen in the tunica albuginea (between arrows). Note a blood vessel of the tunica albuginea between primary and secondary sex cords.

Fig 2—Testis of an 8 day old premature infant. A group of secondary sex cords occupies the outer portion of the tunica albuginea.

solid cords and hollow tubules, but a gradual increase in the number of tubules at the expense of cords may be noted during childhood.

Connective Tissue of the Testis—The connective tissue, as all other constituents of the testis do, shows considerable variation in its development, with the

result that a given normal organ may show a condition of this constituent which is more commonly encountered in somewhat earlier or later stages. Yet it appears that the changes in the connective tissue are, when compared with those in the other tissues of the testis, the most regular and reliable of all if one needs to identify the developmental stage of the organ.

Two portions must be distinguished in the connective tissue of the testis. One consists of the tunica albuginea and the septula which extend between the lobules from the hilus to the tunica albuginea. The other portion comprises the intralobular connective tissue which separates the sex cords or tubules from one another in each lobule. The latter portion contains the interstitial cells. The two portions of the connective tissue will now be described separately, and the description of the interstitial cells remains for the following section. The description is based on sections stained by Mallory's aniline blue method or one of its modifications. The fibers staining blue will be considered as collagenous, and all others, showing distinctly only after silver impregnation, as lattice fibers (reticular argyrophil fibers).

In the testis of the newborn the tunica albuginea contains but a narrow peripheral layer of collagenous fibers. The remaining wider zone, as well as the septula, consists of lattice fibers. The only collagenous fibers in the latter areas are seen in the walls of blood vessels. In the course of the first three months of extrauterine life, increasing portions of the loosely textured lattice fiber layers are replaced by, or transformed into, denser masses of collagenous fibers. At the end of this period the entire tunica albuginea contains densely arranged collagenous fibers, which are now in contact with the lobules of the parenchyma. Only in the vicinity of larger blood vessels may larger collections of lattice fibers be found in the inner part of the tunica albuginea. In a few instances the formation of collagenous fibers in the tunica albuginea takes a slightly different course. A new layer of these fibers appears in the inner portion, and is at first separated from the outer one by lattice fibers. The final result is the same in both cases, and none of the older testes showed two separate collagenous layers.

In the septula, too, a gradual increase of collagenous fibers may be seen during this period, and at the age of 2 months the majority of testes contained definite layers of collagenous fibers in the septula. Here, however, considerable variability is encountered, and large numbers of lattice fibers usually remain adjacent to the collagenous fibers for a long subsequent period. While the septula acquire their collagenous fibers, they decrease in thickness.

The intralobular connective tissue apart from the interstitial cells, which will be described later, shows little change in its structure during the first two weeks after birth. Its cells are densely packed, and no collagenous fibers are contained in it (fig 3). In most testes the sex cords at this stage are separated from one another by connective tissue, although occasional contacts of cords occur in all organs. During the second half of the first month one may notice a tendency toward a looser arrangement of the cells in the intralobular connective tissue. However, the sex cords soon occupy an increasing proportion of the space within the lobules, and at the end of the first month the cords are in contact with one another everywhere (fig 4). (It should be understood that in the condition designated here as contact of cords, a few fibers and the basal membranes separate the cords.) This condition prevails until, during the fifth month, the cords are again separated from one another by an increase in the intralobular connective tissue. This tissue, however, contains relatively few cells and a delicate network of fibers, some of which take a faint stain with aniline blue when treated by Mallory's method (fig 5). This condition is fully developed in most testes of



Figures 3, 4, 5
(See legend on opposite page)

6 to 8 months and persists throughout the period examined, that is, to the age of 12 years or longer

In many of the specimens the cords are closer to each other in the periphery of the organ than near the hilus. The preceding description applies mainly to the peripheral region

Hemorrhages have often been described as occurring in the testes at birth. The present material contains a number of organs of young infants in which part of the intralobular connective tissue or of the septula and loose inner portion of the tunica albuginea is the site of hemorrhages, without apparent damage to the sex cords or the general structure of the organ. Most of these hemorrhages are limited to parts of the organ, and many testes of young infants are entirely free of them on section. It is not correct, as one might be led to believe by Stieve's⁷ description of the testes of newborn infants, that extensive hemorrhage is the rule

The basal membranes of the sex cords also undergo a change during infancy. They can be demonstrated only by impregnation of lattice fibers from early fetal periods on until about two months after birth. At that time they begin to stain faintly with aniline blue, and at the age of 6 months the sex cords of all testes have definite collagenous basal membranes

Interstitial Cells—The large numbers of interstitial cells present in the testes of the fetus and the newborn infant have aroused much interest and speculation. In view of the numerous discussions of this subject in the literature the present account will be limited to a report of the morphologic changes observed. The cells in question will be referred to as interstitial cells from a purely morphologic point of view, without implying that they are strictly comparable in all respects with the interstitial cells of the mature testis. Of all the commonly used staining methods, phloxine and methylene blue stain proved to show the cytoplasm of the interstitial cells best, and all observations reported in the present section are based on this method. In addition to a large body of acidophilic cytoplasm, a large, pale nucleus is found in almost every interstitial cell. Only in a few instances cells were found which contained in the typical cytoplasm small, dark-staining nuclei

Interstitial cells are numerous in the testes of many infants of the first week, and they may form large groups within the intralobular connective tissue (fig 6). It was an exception in the present material to find a testis of the first week with scanty interstitial cells. A slight difference in this respect between full term and premature infants will be discussed in the following section. The

EXPLANATION OF FIGURES 3, 4 and 5

Fig 3—Testis of a 3 day old premature infant (41 cm). The intralobular connective tissue is so cellular that the sex cords do not stand out distinctly with low magnification. The interlobular connective tissue is loose and contains few cells.

Fig 4—Testis of a 2 month old infant. Within the lobules the sex cords occupy a much greater proportion of the space than do those in the preceding figure. The intralobular connective tissue is accordingly scanty. The interlobular connective tissue (septula) is more fibrous than in the early stage.

Fig 5—Testis of a child of 3 years and 8 months. The tubules (former cords) are again separated from one another by larger amounts of connective tissue which, in contrast to the early postnatal condition, is loose and poor in cells. It contains delicate fibers which stain blue with Mallory's aniline blue.

⁷ Stieve, H. Männliche Genitalorgane, in von Mollendorff, W. Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1930, vol 7, pt 2, p 1

number of interstitial cells decreases rapidly during the following weeks and is in most cases greatly reduced at the age of 1 month. Few of these cells remain to reach the second quarter of the first year. After this, the presence of interstitial cells cannot be definitely ascertained with the methods used here, and whatever cells then appear at first glance to be interstitial cells usually turn out to be cells of sex cords or small arteries cut tangentially. The common histologic methods do not allow a decision as to whether or not testes between

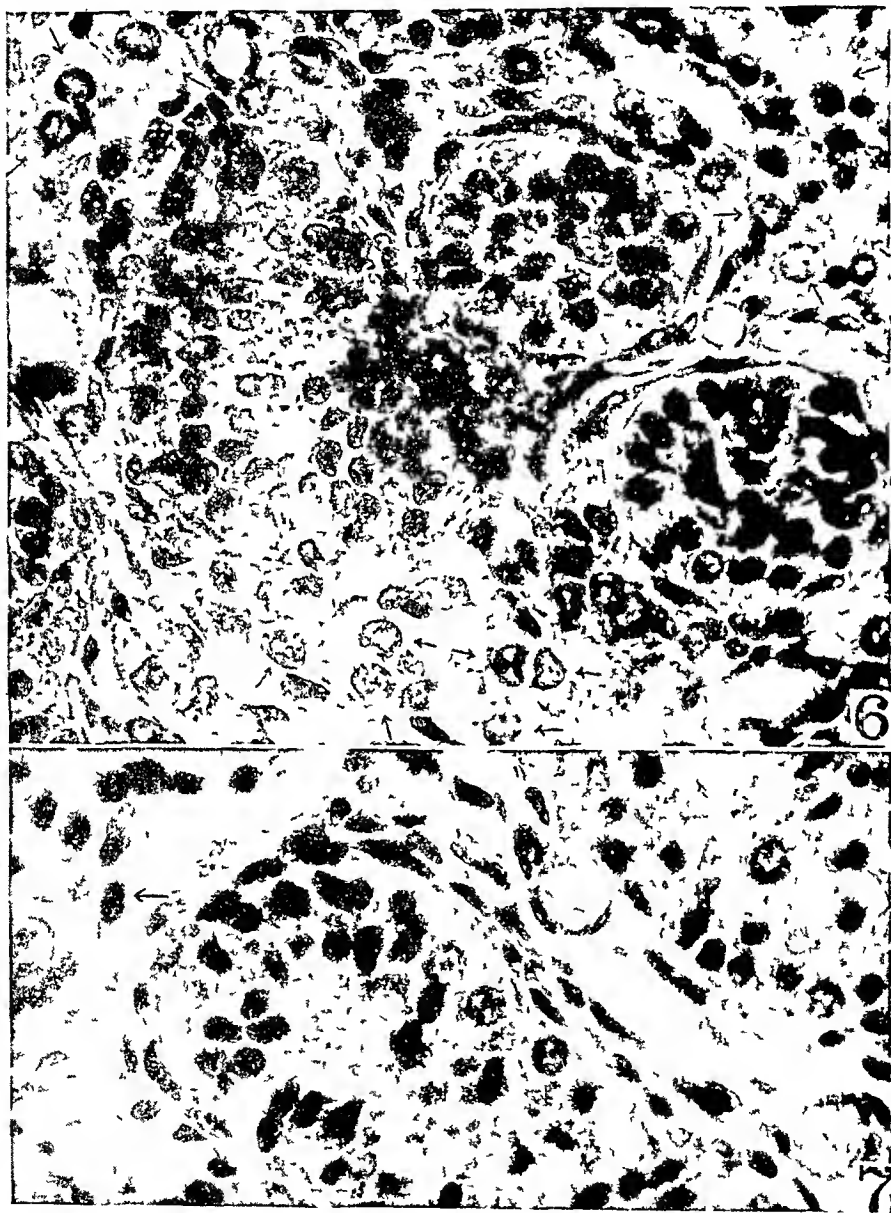


Fig 6—Testis of a 3 day old premature infant (41 cm). The connective tissue between the sex cords contains conspicuous groups of interstitial cells with large, pale-stained bodies of cytoplasm and round or slightly indented nuclei. Some of these cells are marked by arrows.

Fig 7—Testis of a 1 month old infant. The connective tissue contains few interstitial cells. The spindle-shaped cell marked by an arrow may be an interstitial cell in the process of transformation into a connective tissue cell.

the age of about one-half year and puberty contain a few interstitial cells. It must remain for special, perhaps histochemical methods to answer this question.

During the period of disappearance the interstitial cells often appear slender, sometimes spindle shaped, with an intensely staining cytoplasm and oblong, deeply staining nuclei (fig. 7). These may be forms intermediate between interstitial cells and fibroblasts—suggesting, as far as morphologic methods allow such conclusions, that interstitial cells disappear by being transformed into fibroblasts. It is doubtful whether entire cells or parts of their cytoplasm disintegrate. Acidophilic granular masses may sometimes be seen in the intralobular connective tissue, but their interpretation as stages in the necrosis of interstitial cells is uncertain. It should be noted that the disappearance of the last interstitial cells that one can safely discern with the usual methods roughly coincides with the appearance of loose intralobular connective tissue during the second quarter of the first year as described on a foregoing page.

Large numbers of interstitial cells are rarely seen in testes of infants older than 1 month. In the present material a testis of a 2½ month old infant with miliary tuberculosis (not in the testis) showed strikingly large numbers of interstitial cells. There were no other signs of retarded or subnormal development in this testis.

Testes of Premature Infants—Testes of a number of premature infants are included in the present series. It is of considerable interest to see that their development closely parallels that of testes from infants of the same age who were born at term. The number of interstitial cells is often greater in testes of premature infants immediately after birth. This is to be expected since there is normally a decrease in the number of these cells during the last months of embryonic life,⁸ and premature children may be born before this diminution has reached its full extent. However, this difference between premature infants and those born at term is not sufficiently large to be evident in any given case. It is apparent only when numerous cases are reviewed. The difference disappears within a few days after birth, apparently because the more numerous interstitial cells of premature infants disappear just as rapidly as do the less numerous cells of others. No other significant difference between these two groups of infants could be detected in the development of the testes. It thus appears that the timing of early postnatal development of the testes is largely determined by extrinsic factors associated with birth and postnatal life, rather than by the actual age of the infant. In the case of the interstitial cells, and perhaps also in regard to other tissues of the testis, it is probable that a change in the hormonal environment plays a leading role.

Subnormal Testes—It was briefly mentioned before that much of the work on the minute structure of the testis of the infant has been done in order to define "underdeveloped testes" and to determine their frequency which, according to several investigators, is high. Only 3 testes were seen in the present material in which the minute structure differed sufficiently from the standards set in the foregoing pages to be considered as inferior to the normal state. Their ages were 1, 5 and 6 days, respectively (fig. 8). The sex cords were somewhat thinner than usual, and most of them were in the first of the three stages described on foregoing pages. The cells within the cords had a relatively small amount

8 (a) Kitahara, Y. Arch f Entwicklgsmechn d Organ **52** 550, 1923. (b) Neumann, H. O. Ztschr f Geburtsh u Gynak **99** 100, 1930. (c) Diaca, C. Virchows Arch f path Anat **304** 171, 1939. (d) Mita, G. Beitr z path Anat u z allg Path **58** 554, 1914.

of cytoplasm, and their nuclei were seen close to one another. However, these changes were not striking, they were significant only in conjunction with findings in the connective tissue. The cords were separated from one another by a large volume of intralobular connective tissue which showed an abnormally loose arrangement of its elements, being similar to the interlobular tissue of this period. This obscured in many areas the demarcation of lobules. The scarcity of interstitial cells in 2 of these organs further contributed to the impression that the sex cords were located in a tissue of the morphologic properties of interlobular connective tissue. One might conjecture that this was due to failure of the subnormal cords to exert the normal formative stimulation on the connective tissue of the lobule. It is of interest to note that 2 of these 3 testes were from infants with multiple malformations. Their number is too small to allow any conclusions. It should further be emphasized that the condition just described

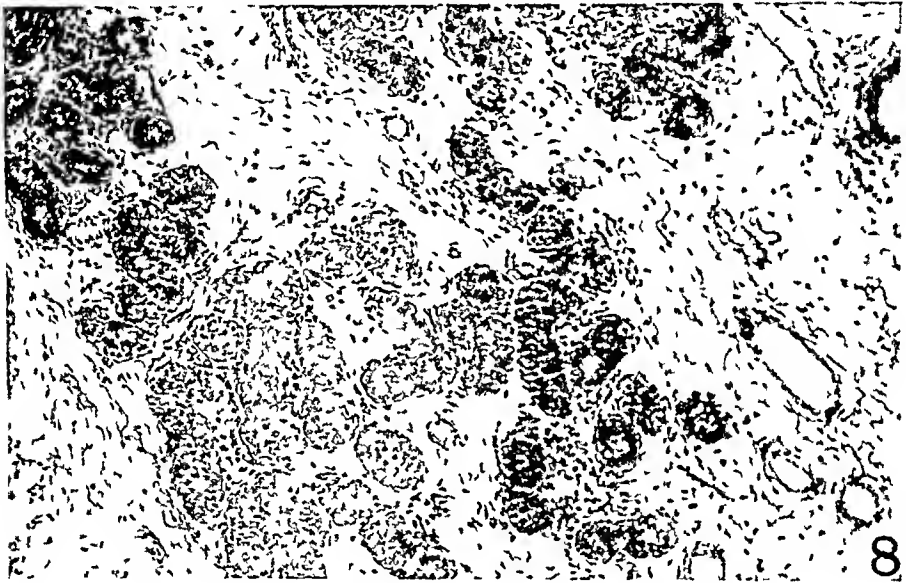


Fig 8—Testis of a 5 day old infant. The sex cords are separated from one another by large amounts of abnormally loose intralobular connective tissue which does not differ significantly from the interlobular tissue. Most of the sex cords are in a relatively immature state. This is an example of a subnormal testis. (For comparison with a normal organ, see figure 3.)

bore no similarity to any earlier stage of normal development and was therefore not an expression of retardation.

There were a few additional specimens in which the structure of the cords and the connective tissue was intermediate between what has just been described as abnormal and the normal state. These might be classified differently by various observers, and part of the discrepancies in the literature are probably due to differences in the classification of the borderline cases. Another difficulty in the recognition of subnormal testes arises when the intralobular connective tissue acquires its loose structure at the age of about 6 months. The normal condition after that time is faintly similar to that in subnormal development, and perhaps this accounts for the fact that the diagnosis of subnormal development of the testes was made here only in young infants. On the other hand, it is possible that the normal condition in older children has in the past been mistaken for subnormal development.

COMMENT

An effort has been made to describe the histologic development of the testes of infants and children and to correlate various changes as closely as possible with definite age periods. The principal difficulty encountered in this study is the wide range of variability in the time at which the organs undergo each phase of their postnatal histogenesis. This variability is probably one of the reasons why many investigators, expecting to see a definite condition at a given time, claim to have found an exceedingly high proportion of abnormal testes. In the present interpretation care was taken to draw the limits of normal stages sufficiently wide to accommodate all organs which would probably have developed into normal testes, that is, the great majority.

The most regular, though perhaps not the most essential, changes in the testes of infants concern the connective tissue. Whenever the question of the age of a given normal testis arises, or of what normal stage corresponds to that of a given abnormal organ, a study of the connective tissue may yield the most reliable information, particularly during the first year of life. The changes in the connective tissue described in foregoing pages are most probably not incidental to the development of the other parts of the testis. It may be assumed that here, as elsewhere, the development of the connective tissue is under the stimulative influence of the morphogenesis of the specific cells of the organ and thus reflects their activity to some extent. The structure of the seminiferous tubules, themselves, or their solid primordia cannot be relied on, because any of the stages described here may occur at any age during childhood.

Finding of intestinal cells in the testes of infants are indicative of the age only to a limited extent. These cells are often present in large numbers shortly after birth, and variations in their number seem to depend on individual differences in the speed of their disappearance as well as on the number present at birth. The former factor is not well understood as yet. A change in the hormonal environment at birth is doubtless of great importance, but it does not account for the long persistence of interstitial cells in some otherwise normal testes. Apart from individual differences caused possibly by variations in the hormone concentration to which the fetus was exposed, the number of interstitial cells present at birth depends on the duration of intra-uterine life, as already pointed out.

The nature and the fate of the fetal interstitial cells, including those persisting in early infancy, are controversial. That these cells are identical with the interstitial cells of the mature testis cannot be taken for granted, it was emphatically denied by Spangaro.⁹ According to

⁹ Spangaro, S. *Anat. Hefte* 1902, no. 18, p. 593.

Kohn¹⁰ and Neuman,^{8b} they develop in the fetus under the influence of hormones of the mother (or the placenta⁷), they diminish as the concentration of these hormones decreases during the latter part of pregnancy,^{8c} and disappear after birth. Several authors¹¹ have reported an increase in the number of interstitial cells in the testes of immature rats after the administration of gonadotropic substances prepared from pregnancy urine or serum. This further supports the assumption that the presence of large numbers of these cells in the fetus is a reaction to its hormonal environment. This is then one of the manifestations of mutual influences of mother and embryo, which were brought under the common heading of synkainogenesis by Kohn¹⁰.

Jaffe¹² held that persistence of numerous interstitial cells with lipid inclusions in infants and children is part of a peculiar developmental disturbance (*Padatrophie*). This concept could not be confirmed in the present material. However, the number of testes over 3 months of age with numerous interstitial cells is too small to allow conclusions.

It is commonly believed that close developmental relations exist between interstitial and connective tissue cells in the sense that each kind may transform itself into the other as need may be.¹³ Hooker¹⁴ recently described interstitial cells developing from connective tissue cells in the testes of young bulls. The present study revealed, during the period of disappearance of many interstitial cells shortly after birth, cell forms which may well be stages by which they are transformed into connective tissue cells.

Several authors¹⁵ have asserted that the great majority of infants—according to Kryle,^{15a} up to 90 per cent—have underdeveloped (*unterentwickelte*) testes. Oberndorfer¹⁶ criticized this view and admitted not more than 20 per cent of underdeveloped testes. It is certainly a misconception of normal structure to decree that only those conditions are normal in which certain supposedly essential parts are

10 Kohn, A. Arch f. Entwicklungsmech. d. Organ. **39** 112, 1914.

11 Moore, C. R., and Price, D. Am. J. Physiol. **99** 197, 1931. Cole, H. H. Am. J. Anat. **59** 299, 1936. Price, D. ibid. **60** 79, 1936. Price, D., and Ortiz, E. Endocrinology **34** 215, 1944.

12 Jaffé, R. Frankfurt Ztschr. f. Path. **26** 250, 1922.

13 Maximow, A. A., and Bloom, W. A Textbook of Histology, Philadelphia, W. B. Saunders Company, 1942.

14 Hooker, C. W. Am. J. Anat. **74** 1, 1944.

15 (a) Kryle, J. Wien klin. Wchnschr. **23** 1583, 1910, Beitr. z. path. Anat. u. z. allg. Path. **60** 359, 1915, Wien klin. Wchnschr. **33** 185, 1920. (b) Voss, H. Centralbl. f. allg. Path. u. path. Anat. **24** 433, 1913.

16 Oberndorfer, S. Die inneren männlichen Geschlechtsorgane, in Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1931, vol. 6, pt. 3, p. 427.

"well" developed according to arbitrary standards. The limits of normal structure can be derived only from unbiased study of large numbers of specimens which would later in life presumably develop into functionally and structurally normal organs. We have no right to assume that 90 or even 20 per cent of all men have abnormal testes, and it must be concluded that the quoted authors have not employed proper criteria. Mita^{8d} stated that much of what Kyrle regarded as underdevelopment was really edema of the intralobular connective tissue.

Diaca^{8c} attacked the same problem experimentally and found that after treatment with hypophyseal hormones the solid sex cords of embryos and newborn animals react with a reversible enlargement. He concluded, contrary to the view of Kyrle and others, that testes with thin cords and much intralobular connective tissue are normal and that those with wide cords merely show a reaction to hormonal stimulation.

Considering the aforementioned variability in the normal development of the testis and the normal occurrence of stages with different relative amounts of intralobular connective tissue during the first year, one finds but few cases of subnormal development as I propose to designate it. One cannot accept either one of the quoted concepts according to which normal testes of infants should contain only wide cords and scanty connective tissue, or the opposite. There remains a small number of testes which show narrow and slightly abnormal cords embedded in large amounts of loose intralobular connective tissue at a time when this does not occur in normal organs, and these may be classified as subnormal. However, it is impossible to give concise criteria of this condition, and various investigators would certainly disagree on some cases. It is misleading to call this abnormal condition underdevelopment, because in the minds of many this term means retarded development. Kyrle, himself, having been challenged by Mita, stated that what he called underdevelopment was an inferior condition, different from any normal stage of development. This might better be designated as subnormal.

SUMMARY

The minute structure of the testis in infancy and childhood is described with regard to changes in the sex cords or seminiferous tubules, the connective tissue and the interstitial cells. It is found that the range of variability is wide, so that it is impossible to correlate definite morphologic stages with closely limited age periods. Of all changes described, those in the intralobular connective tissue are the most reliable when one comes to correlate age and structure of immature testes.

Secondary sex cords, forming groups in the tunica albuginea, occur in normal testes of infants as well as in testes of fetuses

The number of interstitial cells in the testes of newborn infants is variable but usually large. It is perhaps larger in the case of premature infants than in that of infants born at term. However, this difference is not sufficiently great and constant to be regarded as a sign of maturity.

The literature on so-called underdeveloped testes has been critically reviewed and the conclusion reached that if the criteria are properly chosen, the incidence of this abnormality is not nearly as great as has often been suggested.

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RENAL CHANGES ASSOCIATED WITH A CHLORIDE-DEFICIENT DIET IN THE RAT

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THE CHEMICAL changes occurring in tissues and body fluids of the rat fed a diet deficient in chloride have been the subject of previous reports¹ In brief, these changes consist of a lowering of the concentration of blood chloride, an increase of the concentration of blood bicarbonate and an increase of the urinary excretion of citrate, all indicative of chronic alkalosis There is a characteristic shift in the water of the body, in which the apparent extracellular phase is increased in most of the tissues As is the case with most deficiencies, the growth of the rats is stunted The deficient animals attained only about 50 per cent of the weight of the controls when the chloride content of the diet was reduced to between 2 and 5 mg per hundred grams of food The striking renal changes noted previously^{1b} in these rats form the subject of the present report

METHODS

Tissues from the animals used for chemical studies^{1b} were fixed in neutral solution of formaldehyde U S P (1:10) and stained with hematoxylin and eosin In addition, a group of 24 animals was fed the same diet, and the deficient animals, together with the controls, were killed after periods of deficiency varying from six to thirteen weeks Portions of various organs were fixed in Bouin's fluid, and sections were prepared and stained with hematoxylin and eosin A portion of kidney of each animal was fixed in cold acetone, and sections were stained to demonstrate alkaline phosphatase by the method described by Gomori² and to demonstrate calcium by the von Kossa technic

RESULTS

Sections of heart, lung and bronchus, liver, spleen, bone and bone marrow, striated muscle, pancreas, hypophysis cerebri and thyroid, parathyroid and adrenal glands showed no abnormalities (No uniform enlargement of parathyroid glands could be demonstrated, and although some deficient animals showed some thickening

From the Divisions of Pathology and Biochemistry, University of California.

1 (a) Greenberg, D M, and Cuthbertson, E M J Biol Chem **145**: 179, 1942 (b) Cuthbertson, E M, and Greenberg, D M *ibid* **160** 83, 1945

2 Gomori, G Proc Soc Exper Biol & Med **42** 23, 1939

ing and nodularity of the glomerular zone of the adrenal cortex, this was not a uniform finding and could not be demonstrated as a lesion characteristic of deficient animals only) In male rats late in the deficiency (after eighty days) there was complete atrophy of seminiferous epithelium (fig 1 *B*) Earlier (fig 1 *A*), numerous giant cells were present in this epithelium, and stages of arrested spermatogenesis could be demonstrated (forty-two days) No changes were found in the ovary

A uniform lesion was observed in the kidneys Grossly this was a band of yellowish gray material that involved the intermediate zone,³ that is, the collecting tubules In sections stained by the von Kossa method it took on the dark color of calcium (fig 1 *C*) This was the earliest change detectable grossly, although kidneys which appeared normal on gross inspection showed early the microscopic lesions described later As the deficiency progressed the kidney eventually

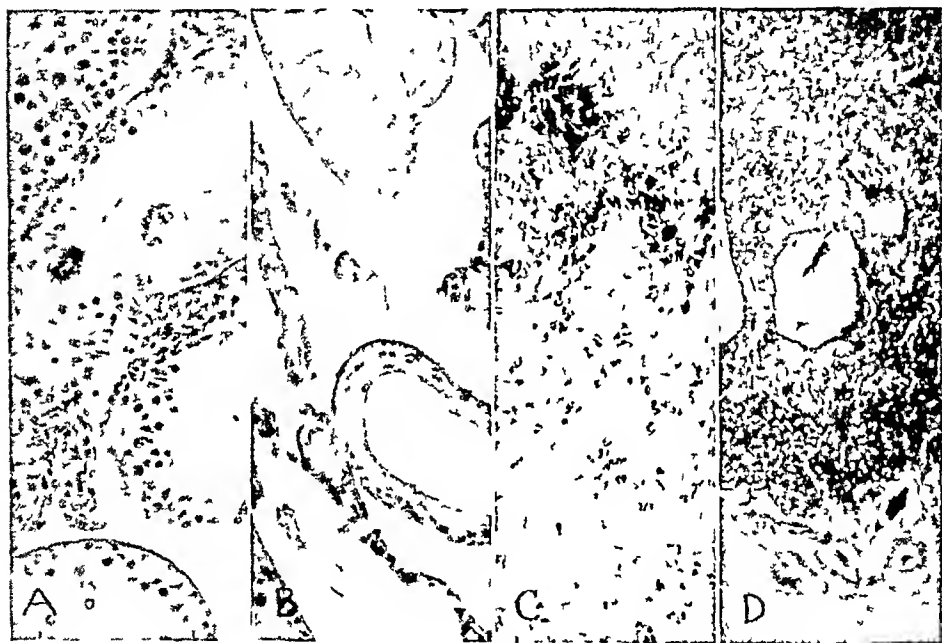


Fig 1—*A* and *B*, stages of testicular atrophy *A*, the forty-two day stage shows giant cell formation, and *B*, the one hundred and forty-two days stage, shows complete degeneration Hematoxylin and eosin, $\times 170$ *C* and *D*, appearance of kidney in rather early (forty-two day) and quite late (ninety-one day) stages of deficiency *C*, shows calcium deposited in the cortical intermediate zone and in the collecting tubules Von Kossa stain, $\times 23$ In *D* the renal pelvis (at the lower right) is intact, however, cortical cysts have been formed by intrarenal obstruction Hematoxylin and eosin, $\times 15$

became a shell filled with fluid, consisting of a much thinned cortex with pelvic epithelium forming folds separated in part from the compressed cortical zone At this stage, the ureters were of usual dimensions, and urine could be readily expressed from the bladder

Histologic renal changes could be traced through a definite evolution, which was not always in strict chronologic order, though early changes were most

3 Kirkman, H Am J Anat 73 451, 1943

frequent in periods of short deficiency, while only advanced lesions were seen after long periods of the diet. This is indicated graphically, in figure 5.

The earliest change is the deposit of granular material, staining as calcium, in the lumen of the connecting and early portions of the collecting tubules (fig 2). At this stage the concentration and the distribution of alkaline phosphatase are entirely normal, that is, the enzyme is in the brush border, in cuboidal cells of Bowman's capsule and near the afferent arteriole⁴. In fact, no histologic changes are identified in any part of the nephron. Shortly after this deposit has occurred, renal epithelial cells in this part of the tubule show degenerative changes, that is, they eventually desquamate, and the tubular lumen dilates. In ulcerated areas a granulomatous response appears now about the foreign material, composed of fibroblasts, lymphocytes and occasionally a giant cell of foreign body type. The progressive changes are shown in figures 3 and 4. This reaction, no doubt, completes the blockage begun by the calcareous deposits, for dilation of

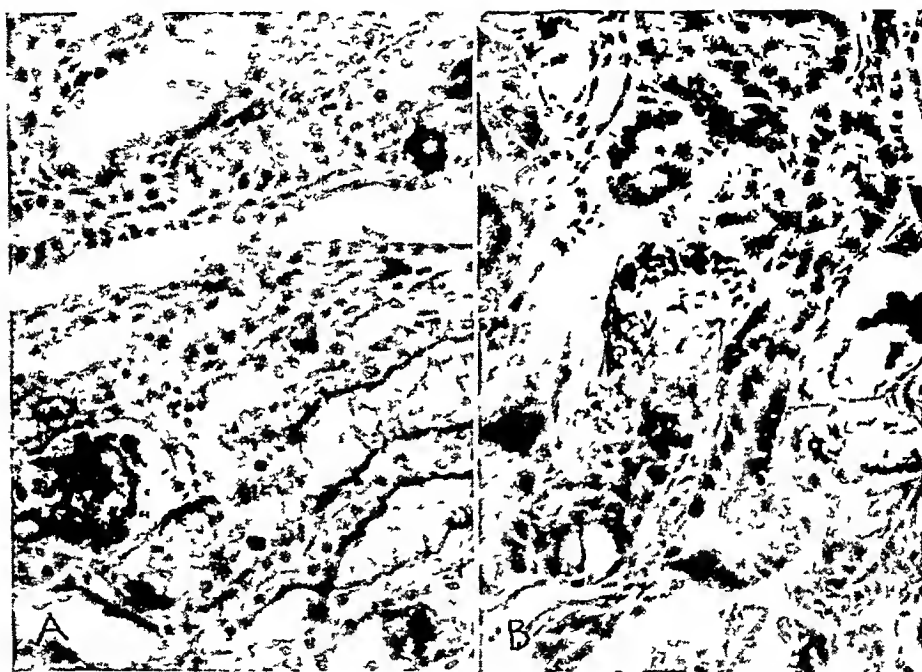


Fig 2—Stages 1 and 2 in the progression of the renal lesion. *A* (forty-two days) shows calcific casts in collecting tubules. Gomori's alkaline phosphatase stain, $\times 230$. *B* (fifty-six days) shows interstitial and tubular reaction (stage 2). Hematoxylin and eosin, $\times 220$.

portions of nephrons behind this region (that is, distal convoluted tubules) soon becomes marked. The proximal tubules remain intact very late in the process, often appearing separated by dilated distal loops and by interstitial fibrous and inflammatory tissue. At this stage alkaline phosphatase is still demonstrable in proximal convoluted loops. The process does not involve nephrons uniformly, and markedly altered units may be separated by single normal nephrons or groups of them. Late in the process the blockage of nephrons becomes widespread and perhaps more nearly complete, so that marked hydronephrosis, intrarenal in origin, results. The shell of cortical tissue, consisting of portions of nephrons proximal to the connecting tubules, shows a widespread granulomatous type of

inflammatory response separating remaining tubules and glomeruli. These tubules and glomeruli are larger than normal—probably a compensatory hypertrophy of functioning units. Bowman's capsule is dilated, the result of late blockage. At this stage alkaline phosphatase has largely disappeared from the proximal tubular epithelium, indicating final functional interference⁵

COMMENT

The foregoing description of the genesis of this lesion is in accord with known facts of renal tubular function. These rats, in chronic alkalosis, show as the earliest change—in fact, within a few hours¹—



Fig 3—Stage 3 extensive granulomatous reaction with dilatation of distal tubules. A group of proximal convoluted tubules at the upper right is intact. Hematoxylin and eosin, $\times 170$. The inset at the lower left shows a detail in the formation of a giant cell ($\times 450$).

a marked drop in urinary excretion of chloride, even very early they excrete an alkaline urine. Since the distal tubule is the site of resorption of chloride⁶ and of water⁷ and the site of urinary acidifica-

⁵ Wilmer, H. A. Arch Path **37** 227, 1944

⁶ Walker, A. M., Hudson, C. L., Findlay, T., and Richards, A. N. Am J Physiol **118** 121, 1937

tion,⁸ the abnormality is reflected in the urine in connecting and collecting tubules. Phosphates⁷ likewise become elevated at this point. The alkalosis and the elevation of phosphates would tend to favor the precipitation of calcium salts. Similar though much less extensive changes are reported in cases of human alkalosis,⁹ probably occurring only with associated dehydration. Hydronephrosis due to long administration of alkali in rats has likewise been reported¹⁰

A somewhat similar histologic reaction has been observed as a response to necrosis of renal tubules in dietary serine damage,¹¹ a similar cellular response to a different injury. However, in the experiment reported here the fact that the normal histologic character and the normal distribution of alkaline phosphatase persists in the proximal

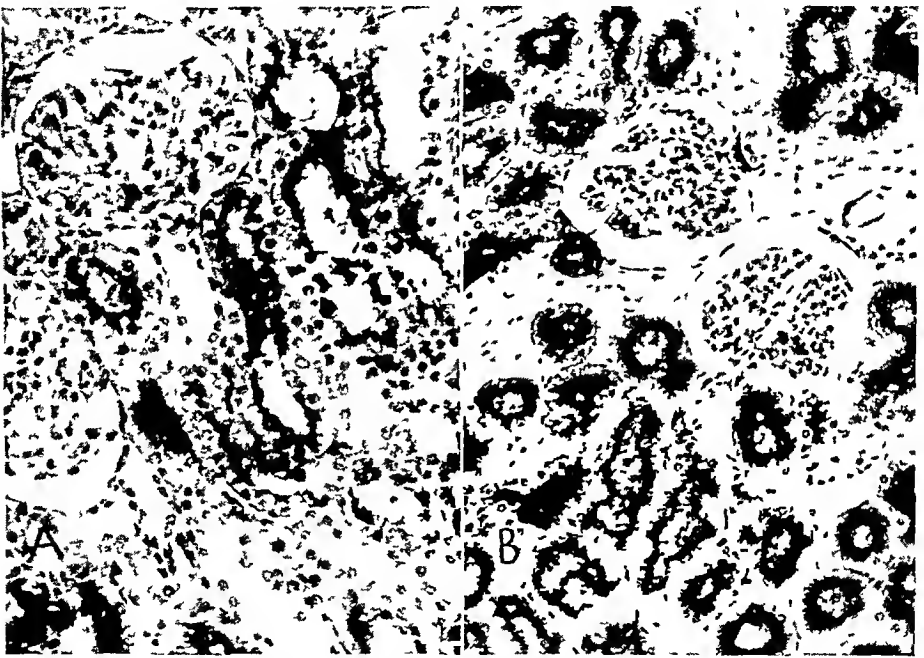


Fig 4—Distribution of alkaline phosphatase late in the process *A*, stage 4, shows ragged alkaline phosphatase in a group of proximal convoluted tubules with absence of phosphatase on the left. Gomori's alkaline phosphatase stain, $\times 225$. *B*, control at forty-two days, shows normal distribution of alkaline phosphatase. Gomori's alkaline phosphatase stain, $\times 170$

7 Walker, A. M., and Hudson, C. L. *Am J Physiol* **118** 167, 1937

8 Montgomery, H., and Pierce, J. A. *Am J Physiol* **118** 144, 1937. Pitts, R. F. *Science* **102** 49 and 81, 1945

9 Kirsner, J. B., Palmer, W. L., and Humphreys, E. M. *Arch Path* **35** 207, 1943

10 Addis, T., MacKay, E. M., and MacKay, L. L. *J Biol Chem* **71** 157, 1926

11 Morehead, R. P., Fishman, W. H., and Artom, C. *Am J Path* **21** 803, 1945, **22** 385, 1946

tubules until very late in the process indicates that the injury occurs by retrograde blockage, producing hydronephrosis, rather than by early degeneration and desquamation of cellular debris. The latter reaction occurs in heavy metal poisoning,¹² in which changes in alkaline phosphatase occur early and much of this material appears in the proximal tubular luminal debris. In fact, here the sequence of changes in the concentration of alkaline phosphatase is like that described in hydronephrosis due to ureteral ligation.⁵

It might be suggested that primary alterations of the distal convoluted tubules, occurring in somewhat the same manner as those described of spontaneous occurrence¹³ but here produced by chemical aberrations, might be expected, since the brunt of the urinary chemical

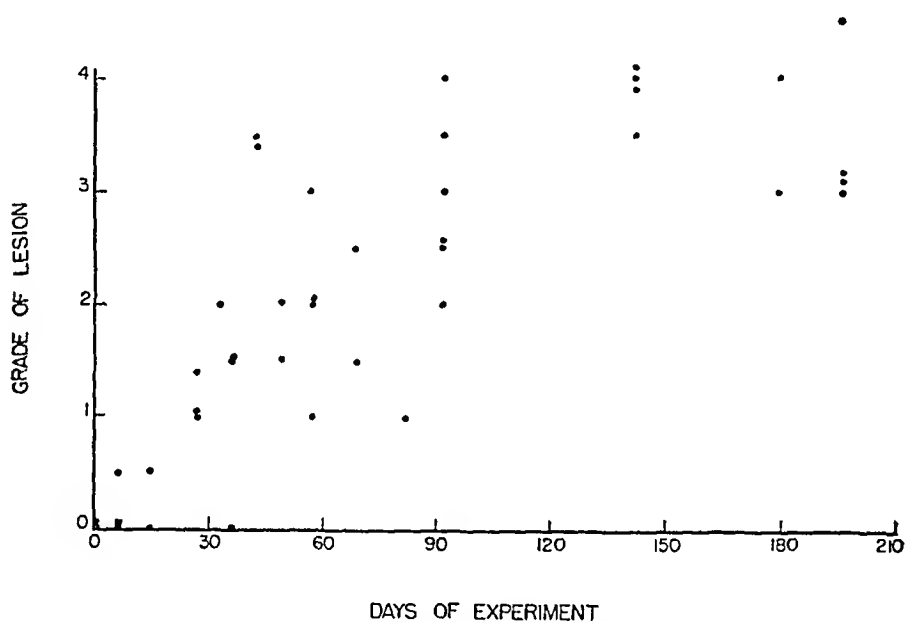


Fig 5—Progression of the lesion as influenced by duration of deficiency

Grade 1 Deposition of calcium in connecting and collecting tubules

Grade 2 The same as grade 1 with the addition of a tubular and interstitial granulomatous reaction

Grade 3 The same as grade 2 with the addition of dilatation of distal convoluted tubules

Grade 4 The same as grade 3 with extensive intrarenal hydronephrosis

The control rats showed no renal changes

abnormality is borne by these units. However, here the obstructive flattening of epithelium precedes any idiopathic histologic change, so that blocking from below better explains the lesion. Since MacNider found this change in old dogs, perhaps in older rats a primary change might occur in distal convoluted tubules.

12 Hepler, O. E., Gurley, H., and Simonds, J. P. Arch Path 39 133, 1945

13 MacNider, W. deB. Proc Soc Exper Biol & Med 55 226, 1944

The lack of complete chronologic regularity in the development of the lesion is to be expected, since other factors, notably the intake of water, would markedly influence the rate of deposition of calcium, dilute urine tending to delay the onset of heavy precipitation. There is, however, a fair degree of regularity in the development of late lesions, which occur only after many weeks of deficiency. So, although there may be variation in the time of appearance of the lesions, all rats suffer some involvement, which then progresses (fig 5)

SUMMARY

A renal lesion uniformly develops in rats maintained on a chloride-deficient diet. Early in the process calcium is precipitated in the lumens of collecting tubules, with consequent blocking and hydro-nephrosis. A granulomatous inflammatory response is a characteristic early reaction to the foreign material and to the degenerating tubular epithelium. Chronic alkalosis is suggested as the basis of the precipitation of calcium.

ANGIOMATOID FORMATIONS IN THE GENITAL ORGANS WITH AND WITHOUT TUMOR FORMATION

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WINSTON-SALEM, N. C.

IN RECENT years a group of neoplasms have been noted in the genital tract which possess an unusual but characteristic morphology. They have occurred most frequently in the epididymis and the testicular tunics, although similar lesions have been found in the uterus and the fallopian tubes. Essentially, they are composed of irregular islands and columns of spherical or oval cells displaying different degrees of vacuolation (fig 1). Coalescence of these vacuolated cells results in the formation of spaces which are lined by cells resembling endothelium and occasionally by cells which have assumed epithelial characteristics (fig 2). In some cases the vacuolated cells constitute the major portion of the lesion, in others they occur singly or in small groups between bundles of fibromuscular tissue (fig 3), which not infrequently shows varying degrees of sclerosis and hyalinization (fig 4). Another feature of these tumors is the presence of a large number of cells resembling lymphocytes, which in some instances are collected into nodules (fig 5). These cells and those showing vacuolation constitute the characteristic features of tumors of this group.

To facilitate the description of the lesions and for reasons given later, they will hereafter be referred to in this paper as angiomatoid.

ANGIOMATOID FORMATIONS IN THE EPIDIDYMIS AND THE TESTICULAR TUNICS

Angiomatoid tumors arising in these structures have been described in the literature under a variety of names: "mixed leiomyoma and lymphangioma,"¹ "lymphangioma,"² "mesothelioma,"³ "adenoma,"⁴

From the Department of Pathology, Bowman Gray School of Medicine of Wake Forest College.

1 (a) Halpert, B. *J Urol* **45** 536, 1941. (b) Malisoff, S., and Helpert, M. *ibid* **50** 104, 1943.

2 Charache, H. *Urol & Cutan Rev* **43** 663, 1939. Foged, J. *Zentralbl f Chir* **66** 1402, 1939. Ugesk f læger **101** 806, 1939. Nakamura, Y. *Mitt a d med Akad zu Kyoto* **26** 812, 1939.

3 (a) Evans, N. *J Urol* **50** 249, 1943. (b) Masson, P., Riopelle, J. L., and Simard, L. C. *Rev canad de biol* **1** 720, 1942.

4 (a) Blumer, C. E. M., and Edwards, J. L. *Brit J Surg* **29** 263, 1941. (b) Gordon-Taylor, G., and Ommaney-Davis, C. *ibid* **29** 260, 1941.

and "low grade adenocarcinoma"⁵ Recently the noncommittal term "adenomatoid tumor" has been suggested⁶

Most of the patients with these lesions have complained of no signs or symptoms other than the presence of an intrascrotal tumor The neoplasms have varied from a few millimeters to 3 cm in diameter and have been either well circumscribed or encapsulated In general, they have presented the gross appearance of fibromyoma The microscopic picture has varied somewhat, but the variations have been due for the most part to quantitative tissue differences rather than to pronounced qualitative alterations of the cells The confusion in terminology has been in part due to these quantitative tissue variations In an attempt to clarify the subject and to facilitate further description, these tumors are divided into several anatomic types

Tumors Composed Predominantly of Fibromuscular Tissue but Containing Scattered Areas of Angiomatoid Tissue—Neoplasms resembling fibromyoma but possessing angiomatoid areas have been described by Halpert^{1a} and by Malisoff and Helpert^{1b} under the name "mixed leiomyoma and lymphangioma" In tumors of this type the fibromuscular bundles constitute the greater portion of the neoplastic structure, the angiomatoid tissue being present only in small amounts

Tumors Composed Predominantly of Angiomatoid Tissue but Containing Fibromuscular Tissue—This type has composed the great majority of the neoplasms of the group under discussion which have been reported⁷ In this type the angiomatoid tissue is surrounded by connective tissue and smooth muscle, which show varying degrees of hyalinization

Tumors Predominantly Angiomatoid and Showing Marked Sclerosis of the Fibromuscular Tissue—No angiomatoid tumors which were characterized by hyalinization and sclerosis have been recorded in the literature One of this type was, however, recently observed in this laboratory Except for a pronounced degree of hyalinization and sclerosis of the fibromuscular tissue, it was similar to the type just described

The patient was a 62 year old white man who was admitted to the hospital with a diagnosis of hypertensive cardiovascular-renal disease On routine examination a small, hard nodule was discovered in the epididymis At operation a small oval tumor was seen in the tail of the gland, and the neoplasm did not appear to be attached to the testicle proper The entire epididymis was removed Examination of the tumor showed a well circumscribed nodule possessing a definite capsule and measuring 1.5 by 1 cm It was hard and composed of a pinkish gray tissue Microscopically, it was seen to contain angiomatoid areas separated by a dense and abundant hyalinized tissue (fig 6)

5 Thompson, G J Surg, Gynec & Obst 62 712, 1936

6 Golden, A, and Ash, J E Am J Path 21 63, 1945

7 Evans^{3a} Masson and others^{3b} Blumer and Edwards^{4a} Gordon-Taylor and Ommaney-Davis^{4b} Thompson⁵ Golden and Ash⁶

ANGIOMATOID FORMATIONS IN THE UTERUS

Angiomatoid tissue of the uterus has been described only in association with well developed fibromyoma. Most authors interested in the more common angiomatoid tumors of the epididymis have completely overlooked the similarity of these neoplasms to those which have

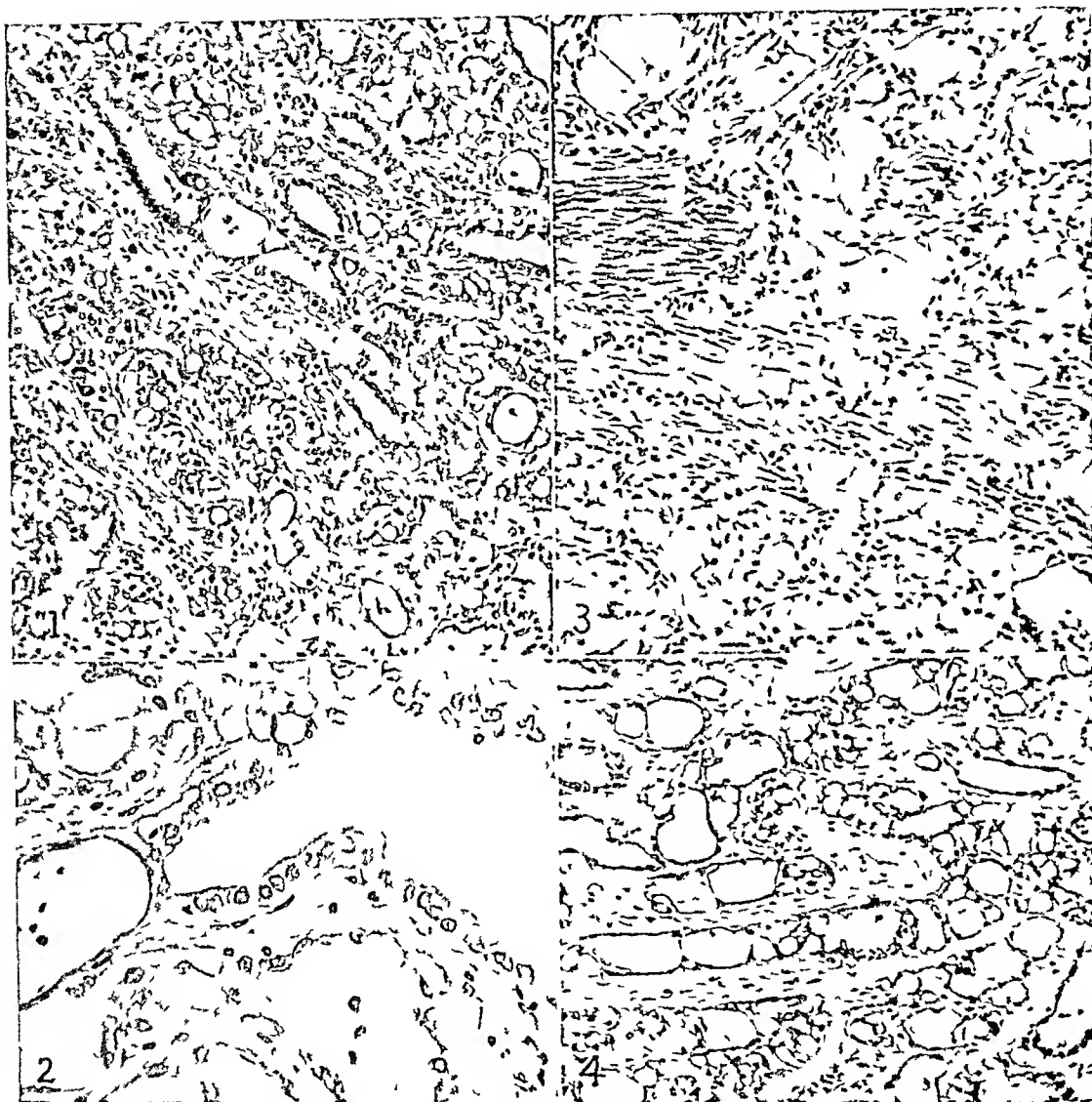


Fig 1—Angiomatoid tumor composed predominantly of angiomatoid tissue. Islands, columns and tubuloalveolar collections of cells showing various degrees of vacuolation and separated by connective tissue bundles which contain lymphocytes and smooth muscle cells. Some of the syncytial masses show beginning vacuole formation, while others have progressed to either the epithelial or the more advanced endothelial stage of differentiation. $\times 100$

Fig 2—The acinus to the right of the photomicrograph is lined by epithelium-like cells, while the wall of the one to the left is composed of endothelium. An intermediate stage is seen in the lower portion of the photograph. $\times 250$

Fig 3—Fibromyoma containing angiomatoid tissue. $\times 100$

Fig 4—An area of hyalinization and sclerosis in a tumor which is predominantly angiomatoid. The majority of the acini are lined by endothelium. $\times 100$

occurred in the uterus Robert Meyer⁸ has described as fibromyoma of the uterus a group of large submucous and intramural tumors which were composed predominantly of myomatous tissue but contained scattered angiomatoid areas Meyer called the neoplasms "lymphangiectatic myomata" Recently Evans⁹ has noted similar changes in a tumor diagnosed as fibromyoma which came under his observation Structurally identical tumors have been described by Curtis¹⁰ and also by Masson and associates^{3b} An excellent illustration of the neoplasms is to be found in Curtis' book¹⁰

ANGIOMATOID FORMATIONS IN THE UTERINE TUBES

Few instances of angiomatoid formations in the uterine tubes have been recorded in the literature,¹¹ and prior to the report of Evans⁹ all of these had been designated as lymphangioma This author reported a "rounded tumor" 8 mm in diameter on the wall of the uterine tube in a white woman 45 years of age and called it "mesothelioma" Microscopically, the lesion was seen to be composed primarily of angiomatoid tissue It is worthy of note that the uterus contained multiple fibromyomatous tumors and a small, firm endometrial polyp It may be significant that, with the exception of the cases reported by Strong,^{11b} all angiomatoid nodules located in the uterine tube have been associated with myoma of the uterus Recently cases of angiomatoid changes in the uterine tubes were reported by Golden and Ash⁶ An additional case which has come under my observation is reported

A Negro woman 44 years of age was admitted to the hospital, complaining of a mass in the abdomen At operation a pedunculated subserous tumor measuring 8 cm in diameter was discovered, diagnosed as fibromyoma The uterus was removed together with one fallopian tube and one ovary Examination revealed, in addition to the uterine neoplasm, a roughly oval nodule 8 mm in its greatest dimension and occupying the greater portion of the wall of the uterine tube The nodule was well circumscribed but not encapsulated and was composed of angiomatoid tissue which extended from the serosa to the epithelial lining of the organ (fig 7)

ANGIOMATOID FORMATIONS IN THE OVARIES

Angiomatoid changes in the ovaries have not heretofore been described A recent case observed in this laboratory therefore deserves special consideration

A white woman 26 years of age was operated on in August 1943, and the left fallopian tube and ovary were removed The ovary had been almost com-

8 Meyer, R, in Stoeckel, W Handbuch der Gynakologie, ed 3, Munich, J F Bergmann, 1930, vol 6, p 237

9 Evans, N Am J Path **19** 461, 1943

10 Curtis, A H A Textbook of Gynecology, ed 4, Philadelphia, W B Saunders Company, 1942

11 (a) Leighton, A Am J Obst **65** 573, 1912 (b) Strong, L W Surg, Gynec & Obst **39** 318, 1924 (c) Masson and others^{3b}

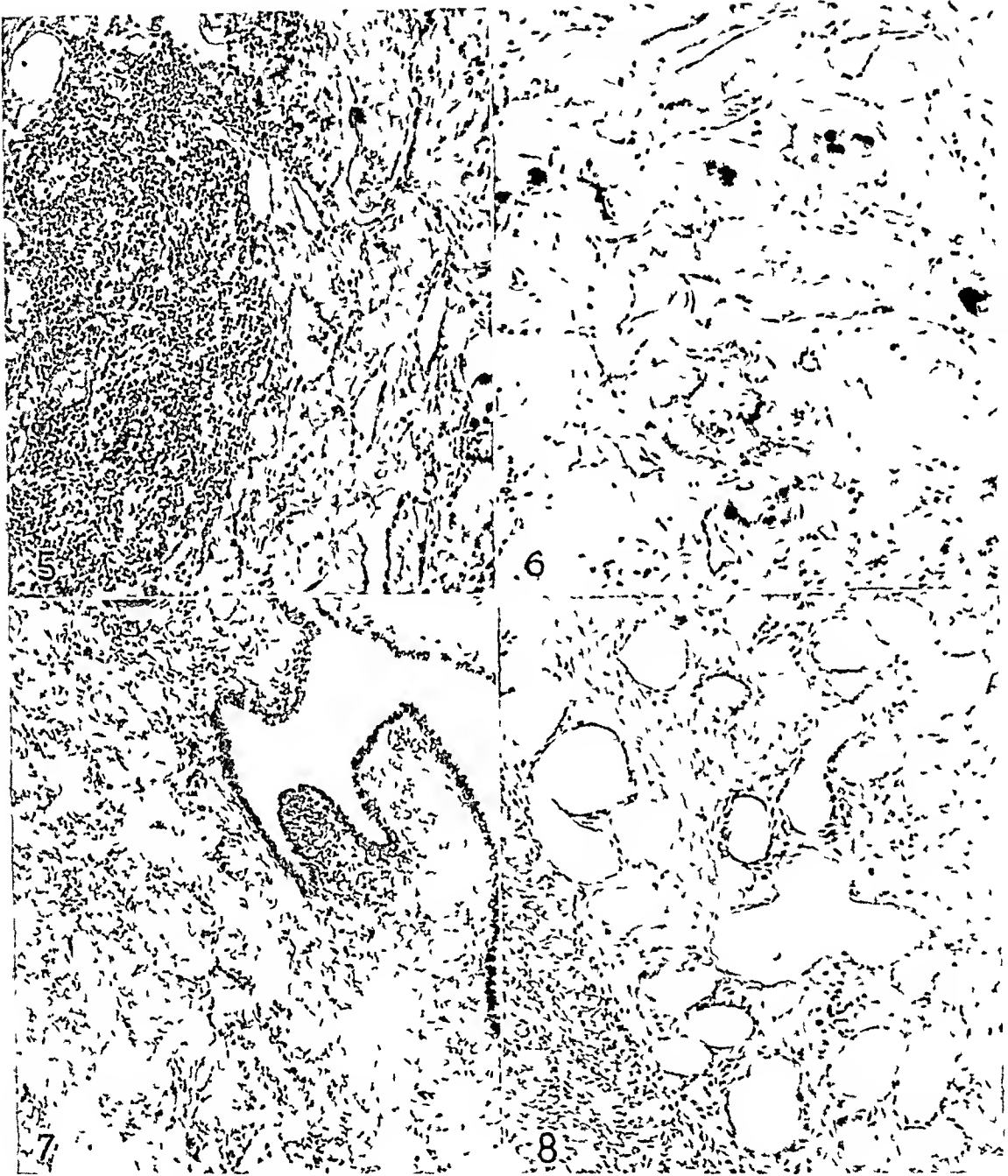


Fig 5—Large collections of lymphocytes in a sclerosed angiomatoid tumor $\times 100$

Fig 6—Angiomatoid tumor of the epididymis characterized by marked hyalinization and sclerosis $\times 100$

Fig 7—Angiomatoid tumor of the uterine tube In the gross specimen the neoplasm appeared to be circumscribed, but note that the angiomatoid changes extended to the epithelial lining of the organ Both endothelial-lined spaces and collections of lymphocytes are seen $\times 100$

Fig 8—Angiomatoid tissue on the surface of the ovary The angiomatoid tissue appears to be arising directly from the cortex of the organ $\times 100$

pletely replaced by a cystic tumor which, after fixation in solution of formaldehyde, measured 6 cm in diameter. The cyst was filled with a clear serous fluid, and a definite epithelial lining could not be demonstrated. No lesion was found in the fallopian tube. The patient made an uneventful recovery, but some time later she began to have abdominal pain. She was again operated on in February 1945, at which time the right ovary was removed. The organ had been almost completely replaced by small cysts containing either clear or bloody fluid and measuring as much as 1.5 cm in diameter. The surface of the ovary was irregular and roughened. Microscopic study revealed that the cortex of the organ had been irregularly replaced by angiomatoid tissue. In some instances this tissue extended to the medulla, while in others it formed irregular accumulations on the surface (fig 8). The germinal epithelial cells were prominent and at times were so arranged as to form adenomatoid structures (fig 9). These, however, showed little anatomic similarity to the angiomatoid areas, and it was evident that the abnormal angiomatoid proliferations originated within the cortex and not from the germinal epithelium of the ovary. It should be emphasized that these changes occurred throughout the organ and that no circumscribed tumor formations were present.

OBSERVATIONS REGARDING THE ORIGIN AND DEVELOPMENT OF ANGIOMATOID TISSUE IN THE GENITAL TRACT

In their early development angiomatoid changes in the genital tract are confined for the most part to the connective tissue in the immediate vicinity of blood vessels, although they occur to a lesser degree within the substance of the fibromuscular tissue (fig 10). The cells which compose this tissue most frequently appear as a syncytium (fig 1), although they are also found singly or collected into small groups. The nuclei are oval and show a coarse reticular network of chromatin. Each possesses a prominent nucleolus and is surrounded by a moderate amount of eosinophilic cytoplasm. In many instances intracytoplasmic vacuoles appear within the more centrally placed cells of the syncytial masses. These in turn coalesce with vacuoles of adjacent cells and this coalescence results in the formation of glandlike lumens. The cells located at the periphery of the syncytium assume epithelial characteristics, while the nuclei of the more centrally placed cells shrink, become hyperchromatic and attain structural features similar to those of the mononuclear cells of the circulating blood.

During the process of luminal formation, and in some instances after it has become complete, vacuoles appear in the more peripherally placed cells of the syncytium (fig 11). These vacuoles coalesce, and the nuclei stain more intensively with hematoxylin. The cellular changes observed thus far are similar to those noted in the more centrally placed cells of the syncytial masses. At this point, however, all of the peripherally placed cells do not proceed to form lymphocytes, some of them assuming structural characteristics similar to those of endothelium (fig 12). Since no basement membrane surrounds the syncytium, the mononuclear cells readily make their way into the

adjacent tissues. The resultant structures are remarkably similar to lymph vessels which are surrounded by a scattering of lymphocytes.

Although in the majority of instances the angiomatoid cells appear in fairly large groups, it must be remembered that they are also seen singly or in small collections. In these instances it appears that vacuolation is followed by the formation of structures resembling lymph capillaries without the intermediary changes seen in the cells of the



Fig 9—Angiomatoid tissue on the surface of the ovary. The germinal epithelium is prominent but apparently plays no part in the formation of the angiomatoid tissue. $\times 100$

Fig 10—Early angiomatoid changes in the wall of a vessel. Both angiomatoid spaces and collections of lymphocytes are seen. Isolated angiomatoid spaces are also present in the fibromuscular bundles. $\times 100$

Fig 11—Vacuolation of the peripherally placed epithelial cells resulting in the formation of angiomatoid structures and isolated lymphocytes. $\times 250$

Fig 12—An endothelium-lined channel. Note lymphocytes in the lumen and also in the wall. One mononuclear cell has a well defined rim of cytoplasm. $\times 250$

syncytial masses Further, in many instances lymphocytes appear to arise singly or in groups directly from the mesenchyme

The mode of development of these angiomatoid lesions suggests a histogenetic relationship to the formation of the vascular system Embryonic tissue destined to form vascular channels first appears in the embryo as solid areas of cells which are called blood islands Tiny vacuoles appear within these mesenchymal cells and rapidly coalesce, forming spaces In this manner the solid areas are hollowed out, the more central cells becoming the earliest blood cells and the more peripheral ones assuming the characteristics of endothelium¹² Prior to the formation of well developed endothelial cells, however, the peripheral cells show at one stage of the process structural characteristics suggesting epithelium A striking similarity is seen, therefore, between the successive stages in the embryonic development of vessels and the formation of angiomatoid tissue

SUMMARY

From the data presented it is evident that the lesions under consideration are composed primarily of two structural units, namely, (1) fibromuscular tissue of the type which ordinarily composes myoma and (2) groups of cells which are predominantly angiomatoid in arrangement and are intermingled with cells structurally resembling small lymphocytes The majority of the tumors of this group which have occurred in the epididymis and the testicular tunics have been predominantly angiomatoid in appearance, although tumors described as myoma containing areas of angiomatoid tissue have been recorded The latter type of neoplasm is the rule in the uterus, and the type which is predominantly angiomatoid has not been described in this organ The angiomatoid nodules which have occurred in the uterine tubes have been small and have not possessed a definite capsule Absence of a capsule is, however, to be expected, since many neoplasms—for example, myoma—do not acquire a capsule until they have reached a considerable size The ovarian lesions seen in the case reported are of special interest, since they occurred as multiple foci and did not produce a definite tumor

A detailed study of the available cases of angiomatoid tumor of the genital tract suggests that the sequence of events in the development of the neoplasm is similar to the formation of vascular channels and lymphoid cells in the embryo These data strongly support the hypothesis that angiomatoid tissue is derived from mesenchymal cells which make imperfect attempts at the formation of lymph vessels and lymphocytes

NOTE.—Drs A H Curtis, C H MacKay and G B Adams gave me permission to examine their material and to incorporate it into this study

HEPAR LOBATUM

Clinical Significance of the Anatomic Changes

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AND

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THAT form of syphilitic cirrhosis of the liver known as hepar lobatum is often attended by symptoms and signs that are identical with those of Laennec's atrophic cirrhosis of the liver, especially in those cases in which the same mechanical factors prevail, such as the mechanisms that produce jaundice, ascites and esophageal varices. In other respects the two diseases may be distinguished one from the other only when it is possible to establish the syphilitic nature of the former and the absence of syphilis in the latter. Anatomically, hepar lobatum is characterized by a greater or less degree of deformity of the liver in the form of coarse and irregularly distributed lobulations due to the overgrowth of connective tissue. The latter is arranged around large groups of lobules and represents either the retracted scars of healed gummas or a chronic productive inflammatory process arising without the intervention of focal granulomas, or both. The surface of the lobulated areas is smooth. The incidence of hepar lobatum is indicated by the fact that the lesion was encountered 50 times in 314 cases of late acquired syphilis occurring among 4,880 necropsies at Bellevue Hospital, or in 16 per cent¹. By this we do not mean to imply, of course, that hepar lobatum is susceptible of diagnosis in 16 per cent of all patients with late acquired syphilis. On the contrary, hepar lobatum may cause no symptoms or signs by which it could even be suspected during life.

Over a period of thirty years hepar lobatum was encountered 102 times among 23,792 necropsies at Bellevue Hospital. According to the clinical records of 28 cases that were available for analysis, the lesion occurred 23 times in males and 5 times in females, 24 patients were of the white race and 4 were Negroes. The average age was 38.5 years, the youngest patient was 19 and the oldest 80. Six patients admitted

From the Laboratories of Pathology, Bellevue Hospital

1 Symmers, D. J. A. M. A. 66:1457, 1916

excessive use of alcohol. The diagnosis of syphilitic cirrhosis of the liver was made during life in 3 cases, and the condition was suspected in 1. In 18 cases the liver was palpable below the right costal slope for distances varying from 2 to 12 cm, in 6 instances the liver was nodular, and in 10 the organ was tender. The spleen was palpable in 13 cases. Jaundice occurred in 8 cases, ascites in 18 and hematemesis in 7. In 12 cases fever was present. The temperature was irregular, varying between 100 and 106 F. The Wassermann reaction was strongly positive in 10. Syphilitic lesions, such as cutaneous gumma, fibrosis of the testicles and penile scar, were noted in 10 cases. From these facts it is apparent that the clinical diagnosis of hepar lobatum depends almost exclusively on such physical signs as jaundice, ascites and residual lesions of syphilis occurring elsewhere and that symptoms are few and of comparatively little importance. The paucity of symptoms

Incidence of Hepar Lobatum by Decades of Life

| Age | Number of Cases | Percentage |
|-------|-----------------|------------|
| 9 | 1 | 0.9 |
| 10-20 | 3 | 2.9 |
| 21-30 | 8 | 7.9 |
| 31-40 | 27 | 26.5 |
| 41-50 | 29 | 28.7 |
| 51-60 | 21 | 20.8 |
| 61-70 | 11 | 10.9 |
| 71-80 | 2 | 1.9 |

and the relative frequency of physical signs are evident in MacCrae and Caven's analysis of the changes in 100 cases of hepar lobatum which were studied from the purely clinical point of view.²

INCIDENCE

The incidence of hepar lobatum by decades of life in 102 cases is given in the accompanying table.

In patients between 30 and 50 years there were 56 cases (54.9 per cent). The lesion, therefore, is one of youth or of comparative youth.

The lesion occurred 83 times in white persons and 19 times in Negroes, a ratio of 4.4 to 1. The proportion of white to Negro patients admitted to Bellevue Hospital is 4 to 1. There were 71 males and 31 females, a ratio of 2.3 to 1. The proportion of male to female patients admitted to Bellevue Hospital is 1.3 to 1. The disease, therefore, is commoner in males than in females and more common in white people than in Negroes.

² MacCrae, T., and Caven, W. R. *Am J M Sc* 172:781, 1926.

In 1927 an abrupt decline occurred in the number of cases of *hepar lobatum* encountered in necropsies at Bellevue Hospital. In view of the fact that about seventeen years earlier preparations of arsenic came into more or less general use in the treatment of syphilis, it seems reasonable to assume that the decline was due, at least in part, to the effects of therapy.

POSTMORTEM OBSERVATIONS

In 102 cases of *hepar lobatum* the following changes were noted at necropsy:

Jaundice occurred in 35 cases (34 per cent), it was slight in 8, moderate in 10 and intense in 17.

Ascites occurred in 32 cases (31 per cent).

Esophageal varices were noted in 15 cases (15 per cent), and in all of them rupture had occurred during life. Unruptured varices, if present, were not recorded.

The state of nutrition was recorded in 40 cases. In 8 cases it was described as "poor" and in 20 as "good", in 12 cases the subjects were said to be emaciated.

Miscellaneous associated lesions of syphilis were noted in 68 cases (67 per cent). In order of their frequency they occurred as follows: syphilitic aortitis in 21, chronic interstitial syphilitic splenitis in 18, fibrosis of testicles in 14—unilateral in 3, bilateral in 11, changes in the bony system in 7, cerebrospinal syphilis in 3, smooth indurative atrophy of the base of the tongue in 2, gumma of lymph nodes, tracheitis and congenital syphilitic pancreatitis each in 1.

Liver—In 37 cases (36 per cent) the liver was coarsely nodular, and in 4 of these the edge of the organ projected beneath the right costal margin, reaching in 2 as low as the level of the umbilicus. In 2 cases the liver was the site of amyloid deposits. In 53 cases the average weight of the liver was 1,556 Gm, a weight which approximates the normal. The smallest liver weighed 608 Gm and the heaviest 3,450 Gm. Solitary gumma was noted in the liver in 7 cases and multiple gumma in 12, in only 3 of these 19 cases did a gumma project above the upper surface of the liver and appear to invite detection during life.

Spleen—In 47 cases of *hepar lobatum* the spleen was described as "normal in size," "slightly enlarged," "moderately enlarged," or "considerably enlarged" at necropsy. In an additional 55 cases the average weight of the spleen was 538 Gm, or 338 Gm in excess of the extreme normal limit of 200 Gm. The smallest spleen weighed 24 Gm and occurred in a 9 year old subject with congenital syphilis. Among adults the smallest spleen weighed 60 Gm and the largest 1,980 Gm. In 18 of the 102 cases of *hepar lobatum* the spleen showed the changes

incident to chronic interstitial syphilitic splenitis, and in 3 cases deposits of amyloid were present

There is a form of splenomegaly which occurs in persons with late acquired syphilis, perhaps exclusively, that is attended by pinhead-sized, ochre-colored bodies consisting microscopically of thickened and hyalinized central arteries with mineralization of their fibromuscular and elastic coats, together with mineral-free hyaline deposits in and around neighboring venules, as well as periarterial hemorrhagic extravasations. It seems that up to 1919 only 9 cases had been described in medical literature—1 each by Simonds,³ Sprunt⁴ and Marini⁵ and 6 by Symmers, Gettler and Johnson⁶, 2 additional cases have since been recorded in the necropsy reports of Bellevue Hospital and 1 case at Goldwater Memorial Hospital. The spleens were enormously enlarged, weighing 1,000, 1,280, 775, 1,900, 3,410, 1,275, 1,050, 1,040, 1,150, 1,020 and 1,030 Gm, an average of 1,357 Gm, or 1,157 Gm above the high normal weight of the spleen in adults. In 1 case the weight was not recorded, but the spleen was described as "three times larger than normal". In this form of splenomegaly it appears that hemorrhage is followed by disintegration of hemoglobin, with iron compounds being deposited in the hyalinized arterial coats, and that the circulation is impaired in such manner as to permit escape of serum, with the precipitation of calcium in fibers that are prepared for its reception as a result of previous deposition of iron. Also, salts of silica and phosphorus are present in increased quantities, the former accounting, apparently, for the ochre color of the deposits in the substance of the spleen. In the 6 cases recorded by Symmers, Gettler and Johnson and in the 3 cases since encountered at Bellevue and Goldwater Memorial hospitals the patients all had syphilitic cirrhosis of the liver or syphilis of the liver and other organs, including fibrosis of the testicles, rupia and like residua.

SUMMARY

From the foregoing observations it is evident that in a certain number of instances hepar lobatum is attended by anatomic changes which are detectable during life and that the incidence of the lesion is such as to warrant a search for it in all cases of late acquired and late congenital syphilis. A history of syphilis and a positive Wassermann reaction or other serologic evidence are important, but signs in the skin or in the visible mucous membranes, fibrotic lesions of the testicle,

3 Simonds, J. P. *J. Infect. Dis.* 5 23, 1908

4 Sprunt, T. P. *J. Exper. Med.* 14 59, 1911

5 Marini, G. *Arch. per le sc. med.* 26 105, 1902

6 Symmers, D., Gettler, A. O., and Johnson, W. M. *Surg., Gynec. & Obst.* 28 58, 1919

changes in the bony system, penile scars and the like, or jaundice associated with an enlarged, firm spleen, with or without ascites, should direct attention to the liver as the possible seat of syphilitic cirrhosis. Finally, in approaching the clinical diagnosis of *hepar lobatum* it would seem, first, that confirmatory evidence should be sought in the form of response to treatment with iodides or other appropriate therapy and, second, that consideration should be given to the advisability of investigating the changes in the liver by the process of biopsy.

BIREFRINGENCE IN TISSUES

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ALTHOUGH students of the finer structure of matter have used the polarizing microscope extensively,¹ there has been little application of the polarizing technic in pathology. This is surprising in view of the ease with which a polarizer and analyzer can be added to an ordinary microscope. Many details not visible in ordinary light are brought into view. Routine methods of preparing sections cause solution of lipids and overshadow inert particles. The double refraction of light by biologic substances is a reflection of the fundamental structure of tissues.

Schmitt¹ distinguished three types of birefringence in tissues.

The first is crystalline birefringence, caused by the regular anisotropic array of the molecules in the substance. It is inherent, and is not altered by changes in the surrounding mediums. Protein, lipid and carbohydrate components show inherent positive uniaxial birefringence, whereas nucleic acid derivatives show inherent negative uniaxial birefringence.

The second type is stream or photoelastic birefringence which results from molecular orientation by external deforming forces. This permits display of birefringence by isotropic solids or liquids. Stream birefringence can be elicited by stirring anisodiametric micelles. The paracrystalline state of tobacco mosaic virus was indicated by its positive birefringence.² Knowledge of contractility was advanced by the demonstration of rotation of molecular links without change in primary valency angles of long chain molecules of myosin and elastoidin.³

The third type is form birefringence, produced by preferential orientation of nonspherical units in a medium of different refractive index. Therefore, it changes with the refractive index of the surrounding mediums and has a zero point. Positive fiber birefringence signifies parallel adlineation of rodlets in the long axis of the fiber. In negative birefringence, platelets, leaflets or disks are oriented perpendicular to

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1 Schmitt, F O. *Physiol Rev* **19** 270, 1939

2 Lauffer, M A, and Stanley, W M. *J Biol Chem* **123** 507, 1938

3 Astbury, W T, and Dickinson, S. *Nature, London* **135** 95 and 765, 1935. Schmitt¹

the long axis of the fiber. Inherent and form birefringence may be found in the same fiber. Apparent negative birefringence can be produced in a fiber by perpendicular orientation of an otherwise positive lipid component. Fibrous structures display linear symmetry, whereas cellular membranes and films show planar symmetry. Anisotropic granules may display radial symmetry, as evidenced by a polarization (maltese) cross in polarized light.

BIREFRINGENCE IN TISSUES

In examination of fresh frozen sections of tissue under polarized light, little alteration is seen in the physicochemical structure of the tissue. The molecular organization producing the refraction is fundamental in the function and the structure of those substances which are locomotive, conductive or supportive. This method of examination also serves as a check on artefacts produced by other methods of examination.

Nervous Tissue—Recently, Witwer, Derbyshire and Corrigan⁴ made a preliminary report on the use of polarized light in the study of tumors of the brain. The fresh myelin sheath exhibits positive uniaxial birefringence with the optical axis disposed radially.⁵ There are concentric lamellas of lipid and neurokeratin protein. The long axes of the protein chains are situated parallel to the surface of the sheath, whereas the lipid molecules are radially oriented. The axon itself displays fiber birefringence. When a nerve is severed, the myelin lipids exhibit changes in polarized light in a few hours. With degeneration, planar asymmetry and anisotropic granules appear. The usual myelin stains will not reveal changes for days.

Muscle—Birefringence in striated muscle has been recognized for many years.⁶ It is positive with respect to the long axes of the fibers. The magnitude of birefringence is greatest in the A (or Q) disk and the Z disk (Klausa's membrane). The I (or J) band is faintly birefringent. During contraction the A disk is reduced in thickness, in stretching it increases. The suggestion has been made that contraction is associated with reduced orientation in the A disk region. Examination of cardiac muscle from human beings, dogs and rats reveals similar birefringence. The intercalated bands are not birefringent. Smooth muscle from the same sources shows much less birefringence.

Collagenous Connective Tissue—This type of tissue displays uniaxial positive birefringence.¹ There is an increase in the birefringence in embryonic fibrous tissue, in developing scar tissue and in areas associ-

⁴ Witwer, E. R., Derbyshire, A. J., and Corrigan, K. E. *Radiology* **41** 130, 1943.

⁵ Schmitt, F. O., and Bear, R. S. *Biol. Rev.* **14** 27, 1939.

⁶ Engelmann, T. W. *Arch. f. d. ges. Physiol.* **11** 432, 1875.

ated with chronic inflammation⁷ Fresh frozen sections of such tissue were subjected to incineration at 550 C for one hour, so that ghost tracings of the tissue were produced Comparison with similar sections from similar, normal, well established areas showed that there was an increase in birefringent crystals in the proliferating tissue The silicon content of connective tissue is the highest of that of normal tissues In the aforementioned sections there was an increase in silicate and calcium crystals The factors of hydration and increased orientation were not measured

The production of a characteristic type of fibrous tissue in dogs by subperitoneal injection of talc has been reported previously⁷ It was observed that the talc crystals tended to adlineate themselves with the long axes of the developing fibroblasts and the subsequent long axes of the collagen fibrils Long chain anisotropic molecules create a field of force in which adjacent micelles are constrained to adlineate The organization by orientation of anisotropic substances which support, protect or move organisms is a result of the asymmetry that produces birefringence In short, asymmetry breeds asymmetry Talc crystals in the presence of developing fibrous tissue act as compasses of the adlineating force

The basket weave laminations found in fibrous nodules are a result of this force Communicating bands may be seen between layers, permitting streaming This is offered as an explanation of how large fibrous nodules may exist with little visible blood supply Polarized light permits distinction of different types of connective tissue without special stains, for each type of fiber has a characteristic pattern

Fatty Tissue—Unmodified fat cells display few birefringent inclusions In the presence of infection, or on exposure to air or to formaldehyde, birefringent specks are visible Foam cells are emulsions in which the cytoplasm is in the diffuse phase If the lipid is birefringent, the foam cell becomes a crystal mass containing myriads of brilliant jewels under polarized light The droplets within a cell are all of the same size

Bone—Positive form birefringence originates from the collagenous matrix which orientates the apatite lamellae⁸ The collagen fibrils run roughly parallel and at right angles to the axis of the haversian canal If the collagen is leached out, the birefringence is reduced, but residual orientation of the apatite micelles may be seen

If calcium deposits from regions affected by inflammation or atheromatous plaques are ground up, flat hexagonal crystals may be seen The

7 Lichtman, A L, McDonald, J R, Dixon, C F, and Mann, F C Surg, Gynec & Obst, to be published

8 Picken L E R Biol Rev 15 133 1940

birefringence is low, and the optical axis is perpendicular to the flat surfaces of the crystals. Granular material which stains blue with hematoxylin is not birefringent.

Hydroxyapatite, the form that calcium phosphate takes in teeth, shows negative intrinsic birefringence and positive structural birefringence. Cementum and dentin also are birefringent.

In the presence of gout, crystals of uric acid and amorphous sodium biurate are deposited, and they provoke a foreign body reaction. The marked birefringence of these crystals makes identification of them easy (fig 1 a). Polarized light also permits positive identification of urea crystals.

Blood—Polarized light of high intensity is necessary to bring out the birefringence of the protein lamins which envelop erythrocytes. Chylomicrons, which show so well under the dark field microscope, are not birefringent.

Chromosomes—The double refraction in chromosomes results from changes in hydration of the chromatin. There are both form birefringence and intrinsic birefringence. The latter is negative uniaxial, resulting from the nucleic acid molecules arranged parallel to the long axes of the chromosomes.⁸ Lines of stress may play a part in the birefringence of asters and spindles.

Skin and Appendages—In conjunction with the electron microscope and roentgen ray diffraction, the spatial arrangement of the long chain protein molecules of keratin has been studied by polarized light analysis. Many practical applications have resulted from knowledge that was gained by studying the effect of stretching, contraction, supercontraction and heat on the protein chains.⁹ Similar methods have been applied in study of the reversible extensibility in synthetic long chain polymers.

Hair, nail and horn exhibit positive uniaxial birefringence of great magnitude. The birefringence of the hair shaft is greatest in the keratinized spindle cells of the cuticle. Dehydration in the distal portion makes the birefringence more prominent. Information gained by inspection of hair under polarized light has attracted the attention of anthropologists, medicolegal experts and those interested in shampoos and permanent waving.

The degenerating keratin in epidermoid cysts loses its birefringence because of hydration, swelling and decay. Pearly bodies in squamous cell carcinoma are markedly birefringent. Nail shows birefringence, with its keratin chains running along the plane of the nail. Sebaceous gland cells show occasional birefringent inclusions.

Eye—The collagen fibers of the sclera are markedly birefringent, the fibers of the cornea are less so. The optic axes of the fibers are parallel to the surface. In the substantia propria of the cornea there

are cross striations of the birefringent substance that is not collagen. Since the optic axes of these fibers are perpendicular to the surface of the cornea, they permit light to enter the optic globe without double

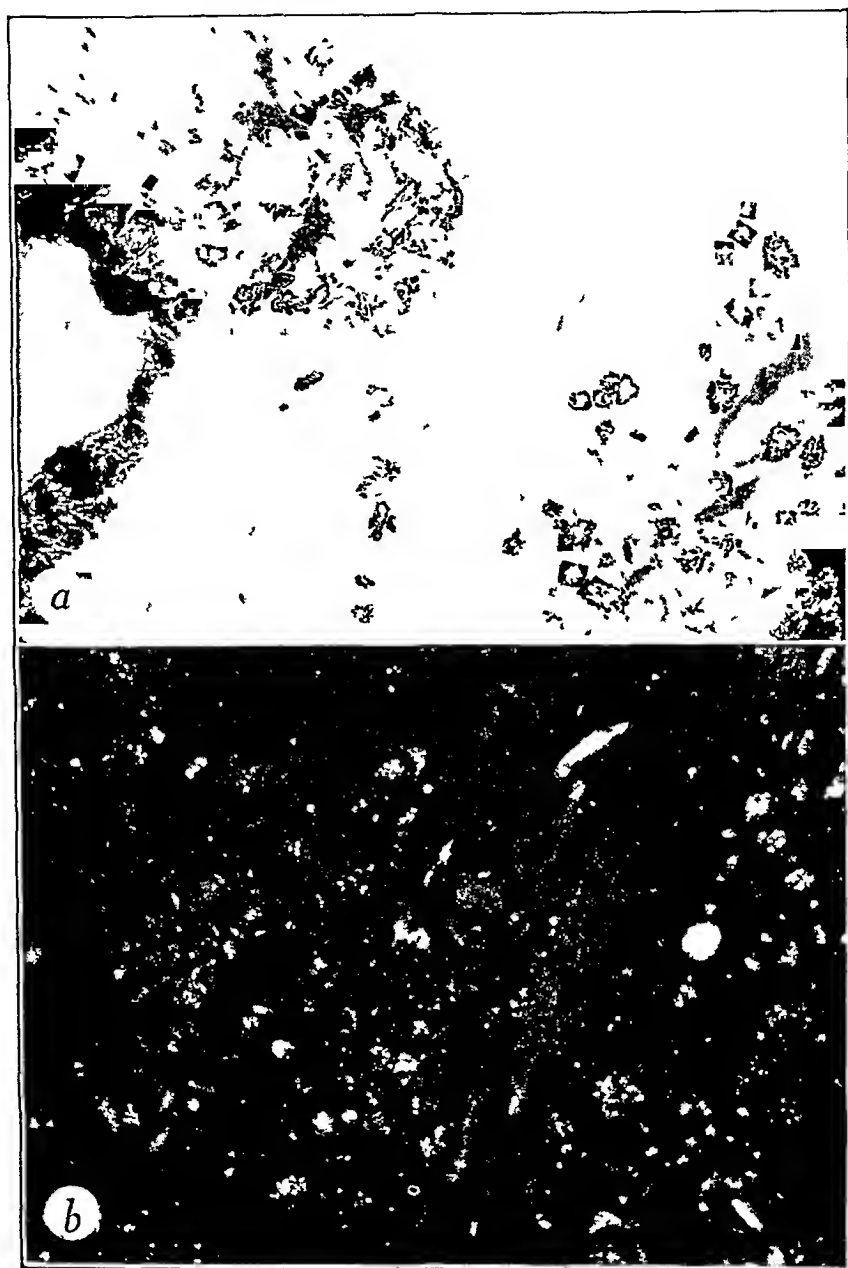


Fig 1—(a) Deposits of uric acid and amorphous sodium biurate found in gout, there is a marked foreign body reaction ($\times 60$) (b) Birefringent crystals in the presence of thyroiditis, found in a colloid goiter. The wide distribution of the crystals suggests that they were factors in the production of the lymphocytic proliferation in this thyroid gland ($\times 60$, partial extinction)

refraction. Where the laminas of the cornea were separated in cutting the sections, these cross striations were absent. We could find no discussion in the literature of the effect of the birefringence of the

cornea, or of what part the cross striations play in preventing it. The normal crystalline lens and the aqueous and the vitreous humor are optically isotropic. The retina, however, contains many structures which display double refraction. There are the myelin sheath and the neural axon, the surface of the erythrocyte and the rod and the cone. However, in the rod and the cone the optic axis is parallel to the long axis of the cell, and light can be absorbed without refraction. These interesting relationships warrant detailed study.

Thyroid Tissue—The parenchymal cells contain birefringent specks. These were too small to identify. The aggregates increase in size in parenchymatous hyperplasia and after treatment with thiouracil. Normal colloid is not birefringent, but colloid of diseased glands shows birefringent crystals. In figure 1 *b* are shown the crystals in a colloid goiter. In regenerative hyperplasia cholesterol crystals and rosettes of fine acicular crystals are seen. Two cases were found in which the masses of crystals had provoked formation of pseudotubercles, so that the gland was first considered to be tuberculous (fig 2 *a* and *b*). It was apparent that the epithelioid and lymphocytic infiltration in some thyroid glands was produced by a birefringent substance elaborated by the thyroid gland.

Lung—Polarized light is useful in the study of the silicotic nodule. Particles between 0.5 and 3 microns do the most damage, obstructing the lymphatic mechanism of disposal. The laminated collagenous nodule is markedly birefringent, and although silica dust is visible in the vicinity, few particles are visible within its confines. However, if the section is incinerated, silica particles that were too small to be visualized are seen within the area of the nodule. Miners who have been exposed to silica dust for a long time also will be found to have silica in the liver and the spleen, for the particles can be transported to the blood stream. Asbestos, a birefringent silicate, produces a distinct variety of pneumoconiosis in which the initial lesion is a cuff of fibrous tissue in a bronchiole. The direction of the progression of the lesion is opposite that followed by silicosis. Talc, another birefringent silicate, causes a self-limited granuloma without progressive fibrosis. In rare instances, discrete fibrous nodules may develop in the lungs of farmers and processors who are exposed to high concentrations of wheat dust for long periods. The cellulose in the wheat dust can be seen, since it is birefringent.

Breast—In plasma cell mastitis, plasma cell and lymphocyte infiltration is found.⁹ Pseudotubercles sometimes lead to an early impression that the condition present is tuberculosis. But polarized light will

⁹ Cromar, C. D. L., and Dockerty, M. B. Proc. Staff Meet., Mayo Clin 16: 775, 1941.

reveal that there are birefringent crystals which produced the characteristic picture. The lesion seems to stem from points where the walls of the dilated ducts have lost their continuity. In some cases the regional lymph nodes showed that some of the crystals had been transported. The crystals were common in the peripheral sinuses.

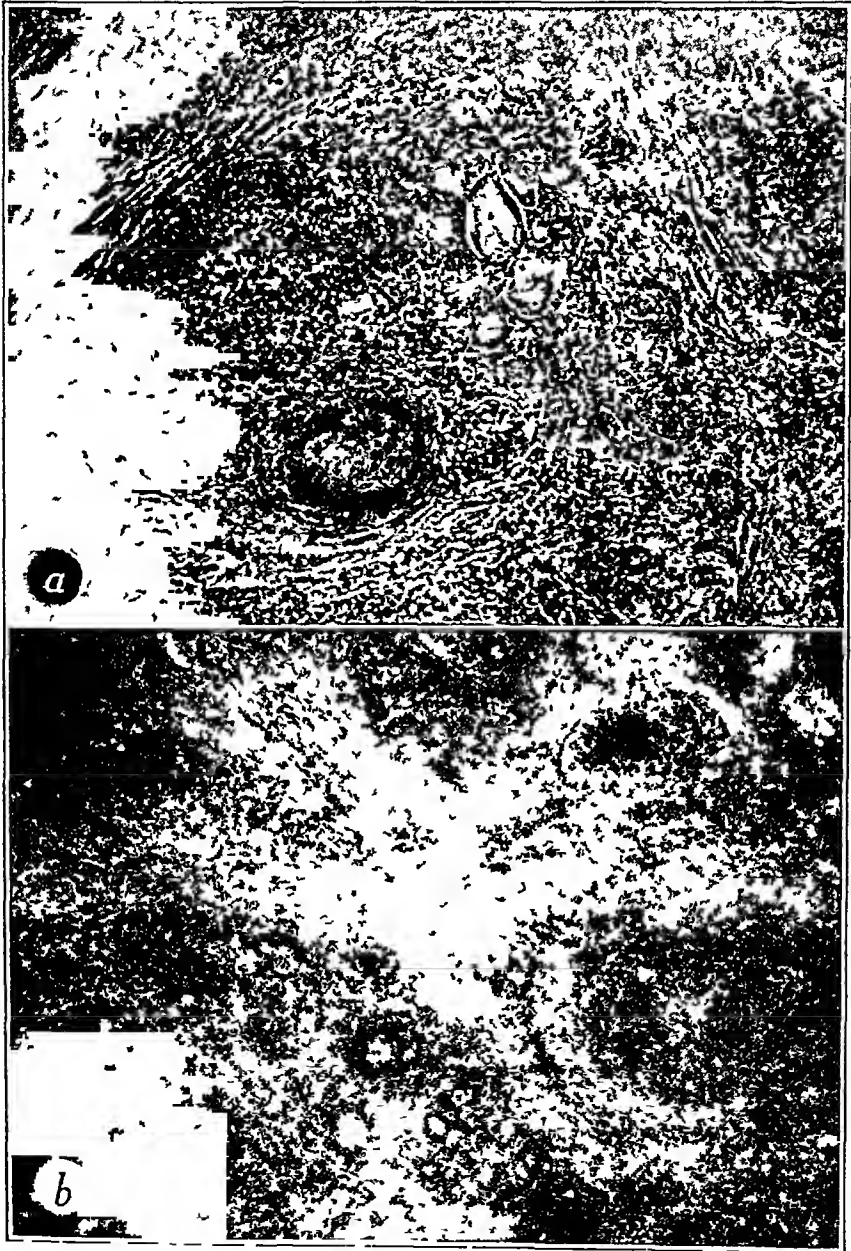


Fig 2—(a) Section of thyroid gland with foreign body reaction to cholesterol esters, which have exerted a foreign body pseudotubercle reaction so closely simulating tuberculosis that the gland was believed to exhibit noncaseous tuberculosis, there were well developed tubercles and extensive fibrosis ($\times 100$, with polarizers at half extinction to show surrounding histologic aspects) (b) Another case in which the histopathologic aspects of the pseudotubercle response around birefringent crystals suggested the diagnosis of "tuberculosis of the thyroid gland" ($\times 40$)

Liver—Although the liver is the site of esterification of cholesterol, the polarizing microscope gives little evidence of this activity. A few birefringent specks are seen in the cells in the center of the hepatic lobule and in the Kupffer cells. This suggests that the cholesterol esters are present in the diffuse phase, and hence that there are no paracrystals. In the presence of cirrhosis of the liver the fibrous tissue is marked by its usual birefringence.

Gallbladder—In cholesterosis or strawberry gallbladder, the enlarged villi are filled with lipid. Some authors have suggested that these break off and form seeds for the formation of cholesterol stones. The masses of amorphous fatty acids are faintly birefringent. Typical cholesterol crystals are abundant. Distinct paracrystals of cholesterol esters are infrequent. They are found only in those gallbladders that exhibit the greatest degree of cholesterosis (fig 3 a). The lipid masses provoke little reaction other than "storage" histiocytosis. But occasionally a gallbladder is seen in which cholesterol ester aggregates have produced a pseudotubercle response. In 1 such case the cholesterol esters had been transported to the hepatic lymph nodes and had produced a picture resembling that of noncaseous tuberculosis.

Gastrointestinal Tract and Peritoneal Cavity—In 198 cases of so-called noncaseous tuberculosis of the ileum specimens were examined, and birefringent crystals were found to be related to the lesion in 49 cases. In 33 cases enough crystals were present to have caused the pseudotubercle reaction.⁷ Where inflammation is present, the connective tissue shows increased birefringence. The action of talc in causing persistence of fecal fistulas and its effect when it is injected subserously into dogs have been reported previously.¹⁰ Both talc and cholesterol esters may produce pseudotubercles in the peritoneum, so that the picture resembles tuberculous peritonitis. In 102 cases the nodules of the peritoneum were subjected to biopsy because tuberculosis was suspected, and in 6 cases the nodules showed talc granuloma, and in 5 cases cholesterol esters were demonstrated to have been the cause of the pseudotubercle response.

Spleen—Where there is an increase in the fibrous tissue of the spleen, it is clearly demarcated by its birefringence. One spleen was observed in which cholesterol esters had provoked a pseudotubercle response. In so-called sarcoidosis of the spleen, markedly birefringent asteroid bodies are present. The crystals have not been identified, but they seem to be the cause of the reaction.

Adrenal Gland—The fasciculate zone of cortical cells exhibits myriads of birefringent spherites. The E compound, F-acetyl, corticosterone and desoxycorticosterone all are markedly birefringent.

10 Lichtman, A. L., and McDonald, J. R. Surg., Gynec. & Obst. 78: 449, 1944.

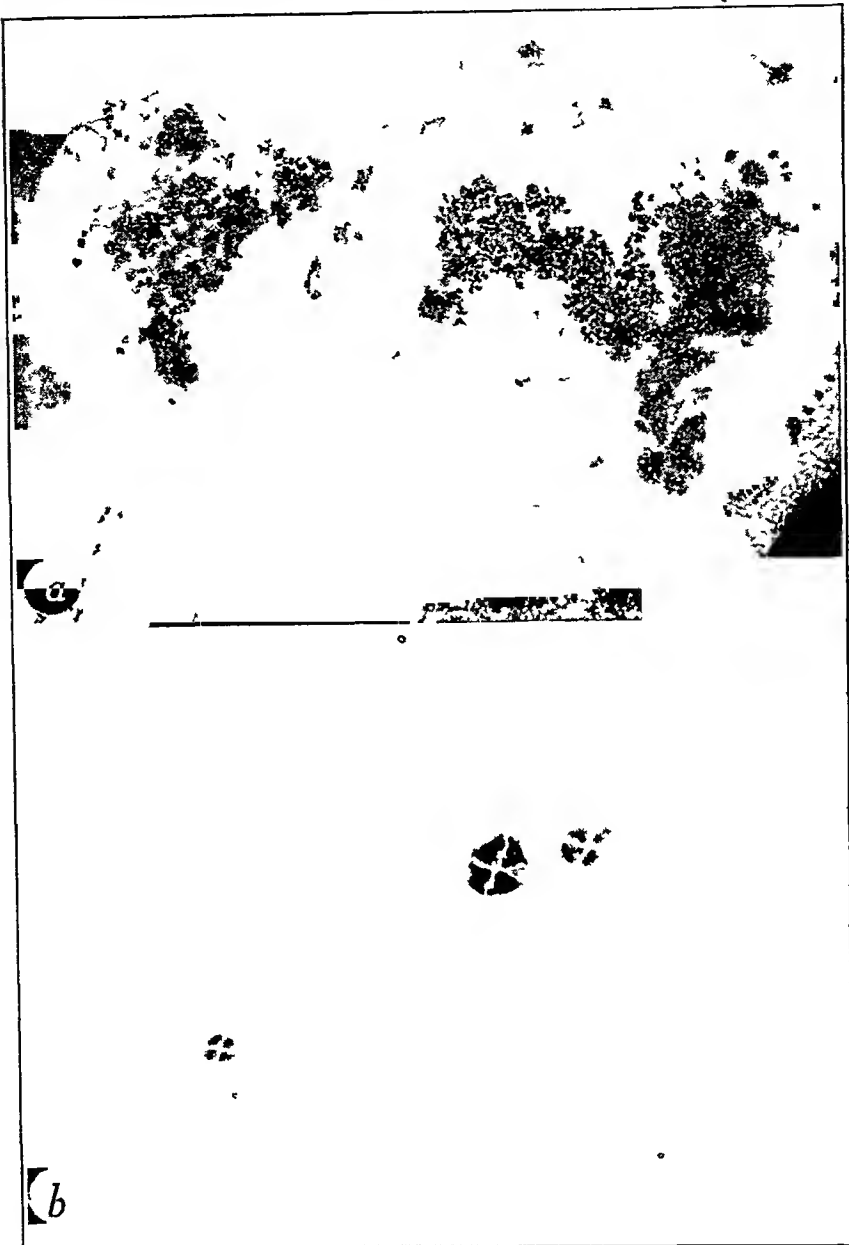


Fig 3—(a) Clubbed villi of a gallbladder, showing cholesterosis. This is a fresh frozen section seen under polarized light at half extinction. The very bright specks are liquid crystals of cholesterol ester, the maltese cross pattern is difficult to photograph, but it shows in some of the droplets. The remainder of the material is uncombined cholesterol and other lipids, when sloughed into the bile these masses conceivably may be seeds for the formation of crystal aggregates or stones ($\times 105$, half extinction). (b) Photomicrograph, at total extinction, of liquid crystal cholesterol esters in the cells of the tubules of the kidney in the presence of so-called lipoid nephrosis, the three liquid crystals show the dark maltese cross formed by four quadrants of refracted light, minute droplets of anisotropic liquids show this polarization cross because of the symmetric radial orientation of the crystallite molecules contained in the droplet. As the physical state changes, an actual rosette of acicular crystals, such as that shown in figure 4, forms. During this transition the granule acquires a foreign body status. When the aforementioned paracrystallite is viewed by the addition of a selenite or gypsum red I crystal, the change in order of the colors in the right upper quadrant and the left lower quadrant identifies the positive birefringence of the cholesterol ester liquid crystal due to structural orientation ($\times 435$, total extinction).

Conference with Dr E C Kendall revealed that the amounts of cortical hormone present in the adrenal gland were not sufficient to permit visualization. The birefringent droplets in the adrenal gland, the ovary and the testis have prompted the suggestion that they are the substance utilized in elaboration of the steroid hormones. Doubt is cast on this by the fact that these droplets are present in organs in which no known steroid hormone is produced.

Kidney—Paracrystalline (liquid crystal) droplets are seen in the tubules of the kidney and in the urine in the presence of lipoid nephrosis (fig 3 b) and to a much less extent in some cases of glomerulonephritis. An attempt was made to study the deposition of cholesterol esters in the kidney. Dogs received a low protein diet, and then blood proteins were depleted by daily plasmapheresis. Hypoalbuminemia, hypercholesterolemia, hypoproteinemia and albuminuria were marked, but cholesterol esters did not appear in the urine. At postmortem examination cholesterol ester droplets were not found in the kidney. The gastrointestinal tract was the site of multiple small ulcers. The experiment was repeated, with the addition of a single dose of 1 mg per kilogram of uranium nitrate, given intravenously. Large numbers of cholesterol ester paracrystals were found in the urine, and at postmortem examination many polarization crosses were seen in the convoluted tubules and Henle loops of the kidney.

Prostate Gland—Granulomatous prostatitis is characterized by an extensive infiltration of plasma cells, large pale mononuclear cells and foreign body giant cells, which replace the normal structure. It has been suggested that the lesion is caused by prostatic secretions that escape into the tissue.¹¹ Examination by polarized light reveals large numbers of fine acicular crystals closely related to the lesion. Staining of the sections with sudan III reveals that one type of crystal absorbs the dye, whereas the other type does not. The former seems to be fatty acid esters, and the latter, nucleotide or phosphatide complexes.

Testis and Ovary—The interstitial cells of the testis and the corpus luteum cells of the ovary show birefringent inclusions. The relationship of these to the functional activity of the gonads is worthy of further study. Examination of ovarian tumors derived from the corpus luteum cells showed myriads of birefringent inclusions.

If a fresh motile human spermatozoon is examined, little can be seen when an ordinary intensity of light is employed. If a light of high intensity is used, or if the specimen is dried or treated with alcohol, birefringence can be seen in the pellicle tip of the head and in the fibrils in the tail.

11 Tanner, F H, and McDonald, J R. Arch Path 36 358, 1943

THE REACTION TO BIREFRINGENT PARTICULATE MATTER

Granulomas produced by bacteria or viruses should not be confused with granulomas produced by particulate matter. Microscopy carried out with polarized light aids in distinguishing between the two. The possibility also must be considered that the original lesion was caused by a micro-organism but that the persistence and the type of lesion are results of the presence of some crystalline material added later. The causative agent may be an abnormal metabolic product, a secretion outside its normal duct system, or a foreign body introduced from the outside. Studies of breast, gallbladder, prostate gland, thyroid gland and ileum carried out under polarized light reveal that a granulomatous lesion can result from the presence of crystals or paracrystals. The size and the nature of the particles determine the lesion. If talc is taken as an example, it will be found that particles less than 0.5 micron in size produce an epithelioid response and that larger particles produce a granuloma, whereas particles more than 10 to 12 microns in size are engulfed in foreign body giant cells without surrounding reaction. Cholesterol esters, finely ground asbestos, and seicite may produce lesions similar to the lesion produced by talc. In experiments embodying subcutaneous injection, much depends on what animal is being used, because tubercles do not develop readily in dogs. Finely divided cellophane and silica will produce progressive fibrosis. Wheat dust will produce a localized fibrous nodule. Fibrous asbestos produces progressive fibrosis in the lung, but if it is ground up and injected, its action resembles that of talc.

Cholesterol esters demonstrate the effect of the molecular arrangement on the response of the tissues. In the paracrystalline state cholesterol esters are tolerated as an innocuous inclusion. So-called liquid crystals exist in cells without the production of a reaction. But when the molecular organization changes, and there is an increase in the orientation of the molecules so that a rosette of acicular crystals forms, the mass becomes a foreign body. The crystals produce a lesion resembling that produced by talc, with the production of pseudo-tubercles.

The physicochemical aspects of cholesterol metabolism are still obscure. Cholesterol metabolism is important because of its relationship to the development of atheromatous plaques in large vessels. In youth, deposited lipids can be reabsorbed. That is, the lipid does not attain to a physical state that will not permit reabsorption. As the age of the person advances, the lipid proceeds beyond the point at which reversal equilibrium can be obtained, just as cholesterol esters, in changing from the paracrystalline phase, become foreign bodies.

It is possible to find cholesterol esters in the presence of any long-standing infection. Although they are products of the infection, they themselves exert an irritant action. For instance, in a study of

granulomatous lesions of the terminal portion of the ileum, a mass of crystals was seen being extruded into the lumen of the intestine (fig 4)

To assist in distinguishing between free and combined cholesterol in sections, it has been suggested that the sections be treated with digitonin and examined under a polarizing microscope. In our experience, the birefringence of cholesterol digitonin has prevented such distinction. Furthermore, the worker cannot depend on solution in lipid solvents to extract these substances. Although the cholesterol esters of short chain or unsaturated fatty acids are freely soluble in alcohol, the solubility of the palmitic and stearic esters at room temperature is about 0.03 Gm per hundred cubic centimeters. For this reason,



Fig 4—Section depicting a possible mode of excretion of cholesterol esters. This is a section of the terminal portion of the ileum of a patient who had an inflammatory granuloma of the ileocecal region. The cholesterol ester mass is being extruded through the mucous membrane. The mucosa and the submucosa contained many particles of cholesterol esters, with a few which had a foreign body reaction around them ($\times 200$, polarizers at half extinction)

and because formaldehyde-precipitated proteins protect the crystals, the worker may see the crystals in routine paraffin sections that have spent hours in lipid solvents.

SUMMARY

Benefits are to be gained by the use of the polarizing microscope in pathology. Polarized light permits visualization of lipid aggregates, crystals and subcellular structures not visible in ordinary light. The anisotropism, or the orientation that produces birefringence in tissues that support, move or protect organisms or conduct nervous impulses, is fundamental in the organization and function of such tissues. In the development of tissues the anisotropic substances or oriented molecules tend to induce mutual adhesion in similar substances adjacent to them.

EXPERIMENTAL ATHEROSCLEROSIS

IX The Effect of Prolonged Feeding of Egg Yolk Powder in Rats

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AND

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ATHEROSCLEROSIS may be produced readily in rabbits by the feeding of cholesterol. The literature on this subject has been reviewed by Duff.¹ In guinea pigs, too, atherosclerosis may be similarly produced, but with more difficulty.² Attempts to obtain these vascular changes in rats, mice, cats, dogs, monkeys, goats, foxes, chickens, pigeons and parrots have been unsuccessful.¹

Hypercholesteremia and atherosclerosis readily develop in the rabbit because this animal is unable to metabolize exogenous cholesterol.

Atherosclerosis may develop in human beings even in the presence of only a moderate increase of blood cholesterol, but it is felt that the lesion produced in the rabbit is not identical with that of human arteriosclerosis. Numerous studies agree with this point of view.³ On the other hand, Leary⁴ suggested that hypercholesteremia hastens the formation of arteriosclerotic plaques in human beings and that the experimental vascular lesion seen in the aorta of the cholesterol-fed rabbit is the counterpart of the lesion found in human tissues.

Some investigators have attempted to produce hypercholesteremia in human subjects by diets rich or poor in fat or by diabetic diets but have either failed or have been only partially successful after long periods.⁵ Corwin⁶ was able to increase blood cholesterol in dogs by

This investigation was aided by a grant from the Oliver Rea Fund.

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1 Duff, G L. Arch Path **20** 81, 1935.

2 Bailey, C H. Proc Soc Exper Biol & Med **13** 60, 1915.

3 Page, I H, Kirk, E, and Van Slyke, D D. J Clin Investigation **15** 109, 1936. Lande, K E, and Sperry, W M. Arch Path **22** 301, 1936. Page, I H. Ann Int Med **14** 1741, 1941. Elliot, A H, and Nuzum, F R. Arch Int Med **57** 63, 1936. Hunt, H M. New England J Med **201** 659, 1929.

4 Leary, T. Arch Path **21** 459, 1936, J A M A **105** 475, 1935.

5 Gardner, J A, and Gamsborough, H. Biochem J **22** 1048, 1928. Steiner, A, and Turner, K B. J Clin Investigation **19** 373, 1940. Bruger, M, and Somach, I. J Biol Chem **97** 23, 1932.

6 Corwin, W C. Arch Path **26** 456, 1938.

feeding them lecithin for six weeks Steiner and Domanski⁷ confirmed these findings in dogs and human subjects, using egg yolk powder as the source of lecithin Sperry, Jailer and Stoyanoff⁸ also demonstrated that the serum cholesterol level may be elevated in monkeys after three days' feeding of egg yolk Steiner and Domanski⁷ showed that feeding of egg yolk powder to patients in addition to a high caloric diet for six to ten weeks resulted in a rise in serum cholesterol and in body weight⁹ A similar diet increased the serum cholesterol in dogs without the production of atherosclerosis or fatty infiltration of the liver and other organs⁷ In monkeys the feeding of two to five eggs or egg yolks failed to alter the blood cholesterol level during the following twenty-four hours On the other hand, an increase was noted in the free cholesterol fraction and especially in the cholesterol esters when the egg feeding was continued for an additional three days⁸

Since diets enriched with egg yolk have been shown to produce hypercholesteremia in man, monkeys and dogs without vascular sclerosis, this investigation was extended to include white rats

MATERIAL AND METHOD

Ten male white rats weighing 240 to 278 Gm were employed in this study Six rats were fed ad libitum a paste mixture¹⁰ of egg yolk powder and ground Purina dog chow checkers in the ratio of 1:4 for three months and then a paste of pure egg powder¹¹ alone for the next six months The remaining 4 rats served as controls and were fed only Purina dog chow checkers¹² In both groups drinking of water was freely permitted The animals seemed in perfect health throughout the experimental period, ate well and gained weight regularly

At monthly intervals the systolic blood pressure of each animal was determined by the method of Williams, Harrison and Grollman¹³ At regular intervals the animals were weighed, and blood was withdrawn by cardiac puncture for the determination of blood cholesterol Cardiac aspiration caused the death of 3 experimental animals at three, six and eight months, respectively Whole blood

7 Steiner, A, and Domanski, B *Am J M Sc* **201** 820, 1941

8 Sperry, W M, Jailer, J W, and Stoyanoff, V A *Federation Proc* **1** 135, 1942

9 Steiner, A *J A M A* **116** 2752, 1941

10 Analysis (by Elsie V Frost, B A, New York Post-Graduate Hospital) showed total lipid 33 per cent, fatty acids 31.8 per cent, total cholesterol 1.2 per cent, cholesterol esters 0.8 per cent, lipid phosphorus 0.3 per cent and lecithin 7.8 per cent

11 Analysis (by R C Williams & Co, New York) showed lecithin 14.4 per cent, vitellin 31.6 per cent, nuclein 3 per cent, cerebrin 0.6 per cent, glycerophosphoric acid 2.4 per cent, cholesterol 8 per cent, fats 40.6 per cent, coloring matter 1 per cent, salts 2 per cent and water 3.6 per cent

12 Analysis (by Ralston Purina Co, St Louis) showed protein 22.5 per cent, fat 5.5 per cent and carbohydrates (nitrogen-free extract) 50.25 per cent

13 Williams, J R, Jr, Harrison, T R, and Grollman, A *J Clin Investigation* **18** 373, 1939

was analyzed for cholesterol by Sackett's modification of Bloor's method¹⁴ At the end of nine months, the remaining 3 experimental and 4 control animals were killed by a blow on the head, and sections of the aortas, livers, kidneys, spleens and lungs were taken for gross and microscopic study Microscopic sections of tissues fixed in Bouin's solution were stained with hematoxylin-eosin For qualitative estimation of fat content, tissues were fixed in solution of formaldehyde U S P and stained with sudan III

In addition, the livers were analyzed¹⁵ for total cholesterol and lipid phosphorus, the latter was determined by the Whitehorn method¹⁶ For these analyses, the livers were cut into small slices, and 20 cc of 25 per cent potassium hydroxide was added for each gram of wet liver Hydrolysis was carried out on an electric hot plate for three hours, and then the material was cooled Extraction was done in a separatory funnel, four successive 100 cc samples of ether being used Each of the four ether fractions was washed with the same 10 cc portion of water All of the ether samples were combined and evaporated to dryness Whenever the residue was colored, it was reextracted with purified benzine U S P and again evaporated to dryness The residue was taken up in 20 cc of chloroform, and 1 cc of the preparation was used for analysis

RESULTS

The chart illustrates the changes observed in whole blood cholesterol, systolic blood pressure and body weight during the prolonged feeding of egg yolk powder to 6 white male rats, which were compared with 4 control white male rats fed a normal diet In the experimental group the whole blood cholesterol was elevated throughout most of the observation periods, the greatest increase occurring in the last four months

The blood pressure was only slightly elevated in each group during the early months of the study and showed a gradual but definite decline toward the end of the experimental period

Both the experimental and the control animals gained weight at approximately the same rate throughout the study In the last few months the average gain of the rats fed egg yolk was slightly greater than that of the control rats However, during the first three months, when the experimental animals received the mixed diet (egg dog chow checker-1 4) the rate of change of the whole blood cholesterol and of the body weight was less notable

Necropsies resulted in the following observations After three months of experimentation 1 rat revealed neither gross change in any of its organs nor microscopic evidence of increased deposition of fat in the liver However, 2 rats examined grossly after six and eight months, respectively, showed slight to moderate increase of fat in the perirenal tissues, in the subcutaneous tissues and in the liver, no other changes were demonstrable in any of the other organs, including the aorta Microscopically, too, there was an increase in hepatic fat deposits At the completion of nine months all the rats fed egg yolk revealed markedly increased storage of fat in the subcutaneous depots, the mesentery, the perirenal areas and the liver, none was evident in the other organs, including the aorta Special study of the latter failed to show any atherosclerotic plaques Microscopically, these findings were confirmed and large amounts of fat were found deposited in the

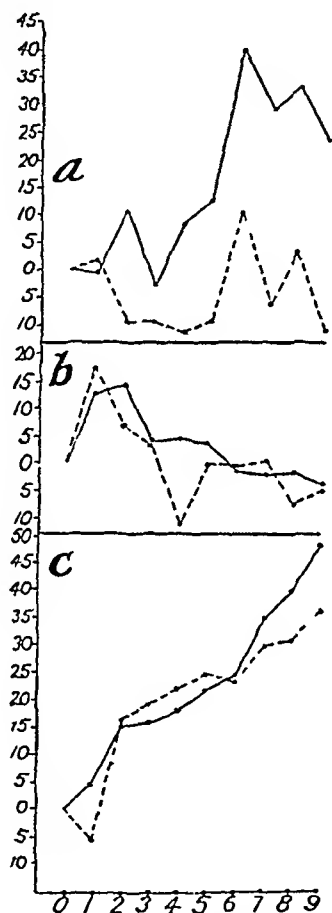
14 Sackett, G E J Biol Chem 64 203, 1925

15 With the technical assistance of Samuel Member, B S, New York Post-Graduate Hospital

16 Whitehorn, I C J Biol Chem 62 133 1924-25

portal areas of each hepatic lobule while none was evident in the aorta. In the rats fed a normal diet no gross or microscopic changes were demonstrated.

In 4 control and 3 experimental rats the cholesterol and lipid phosphorus contents of the wet liver were determined. The cholesterol content of the liver averaged 260 mg per hundred grams in the control animals and 672 mg per hundred grams in the experimental rats. The lipid phosphorus content of the liver calculated as lecithin¹⁷ averaged 17 mg per hundred grams in the control and 14 mg per hundred grams in the experimental animals.



Average percent change in (a) whole blood cholesterol, (b) systolic blood pressure and (c) body weight in 6 white rats fed egg yolk powder (solid line) and 4 controls fed a normal diet (broken line). The numbers at the left represent percentages, those at the bottom, months.

COMMENT

In the present study, in which rats were fed egg yolk powder for prolonged periods, blood cholesterol was increased over the initial level without the development of atherosclerosis. It is possible that lecithin, which has been shown to produce hypercholesteremia,⁶ inhibits the

¹⁷ Mattice, M. R. Chemical Procedures for Clinical Laboratories, Philadelphia, Lea & Febiger, 1936, p. 94.

deposition of cholesterol in the aorta because of its choline content. This point of view has been maintained by Steiner,¹⁸ who suggested that choline causes reabsorption of atheromatous lesions produced in the aortas of cholesterol-fed rabbits. Steiner¹⁹ and Kesten and Silbowitz²⁰ observed that choline delayed the production of, or partly prevented, atherosclerosis in rabbits. In addition, Meeker and Kesten²¹ were able to diminish the incidence and the degree of atherosclerosis in cholesterol-fed rabbits by feeding a high protein diet of soybean flour. On the other hand, Baumann and Rusch²² and Himsworth²³ found that choline did not prevent deposition of cholesterol in the aortas of rabbits fed cholesterol.

So far as deposition of fat in the liver is concerned, this investigation demonstrated a marked increase of fat in the portal areas of each lobule and a quantitative increase of cholesterol in the liver. This confirms the findings of earlier studies²⁴. On the other hand, it has been shown that lecithin and choline²⁵ have definite lipotropic properties and that yeast or other rich sources of the vitamin B complex added in large amounts to the diet protected against or produced clinical improvement in cirrhosis²⁶. Other investigators have pointed out that lack of certain food factors such as those found in yeast caused impairment of function as well as fatty and cirrhotic changes in the liver²⁷. Furthermore,

18 Steiner, A. *Proc Soc Exper Biol & Med* **39** 411, 1938

19 Steiner,¹⁸ p 231

20 Kesten, H D, and Silbowitz, R. *Proc Soc Exper Biol & Med* **49** 71, 1942

21 Meeker, D R, and Kesten, H D. *Arch Path* **31** 147, 1941, *Proc Soc Exper Biol & Med* **45** 543, 1940

22 Baumann, C A, and Rusch, H P. *Proc Soc Exper Biol & Med* **38** 647, 1938

23 Himsworth, H P. *Acta med Scandinav (supp)* **90** 158, 1938

24 Chalutow, S S. *Virchows Arch f path Anat* **207** 452, 1912. Amitschkow, N, and Chalutow, S S. *Centralbl f allg Path u path Anat* **24** 1, 1913. Bailey, C H. *J Exper Med* **23** 69, 1916. McMeans, J W. *J M Research* **33** 475 and 481, 1916. Kimura, T. *Tr Jap Path Soc* **21** 370, 1931. Okey, R. *J Biol Chem* **100** 144v, 1933

25 Best, C H, Hershey, J M, and Huntsman, M E. *J Physiol* **75** 56, 1932. Best, C H, and Huntsman, M E. *ibid* **75** 405, 1932. Best, C H, Mawson, M E H, McHenry, E W, and Ridout, J H. *ibid* **86** 315, 1936. Kesten and Silbowitz²⁰. Himsworth²³

26 Von Glahn, W C, and Flinn, F B. *Am J Path* **15** 771, 1939. Kensler, C J, Sugiura, K, Young, N F, Halter, C R, and Rhoads, C P. *Science* **93** 308, 1941. Patek, A J, Jr, and Post, J. *J Clin Investigation* **20** 481, 1941

27 Lillie, R D, and Sebrell, W H, in *The Pathology of "Yellow Liver" of Dogs*, National Institute of Health Bulletin no 162, Treasury Department, Public Health Service, 1933, p 23. Rhoads, C P, and Miller, D K. *J Exper Med* **67** 463, 1938. Gyorgy, P, and Goldblatt, H. *ibid* **70** 185, 1939. Rich, A R, and Hamilton, J D. *Bull Johns Hopkins Hosp* **66** 185, 1940

Blatherwick and his co-workers²⁸ showed that the fatty liver observed on feeding rats raw liver, dried whole liver or cooked whole eggs resisted the lipotropic action of lecithin

To further complicate the picture, Himsworth and Glynn²⁹ described two pathologic states of the liver which were produced by dietary means first, massive hepatic necrosis like acute yellow atrophy due to the lack of a protein component and prevented³⁰ by the addition of casein or methionine but not by that of cystine or choline and, second, diffuse hepatic fibrosis like portal cirrhosis resulting from long-continued fat infiltration of the liver and inhibited by²⁹ the administration of yeast and choline. These workers further pointed out that most previous studies of hepatic damage caused by dietary insufficiency and of the effects of lipotropic substances thereon were confusing because of the failure to recognize two distinct pathologic states

It is possible that fat was deposited in the livers of the rats used in this study because the diet, which consisted wholly of egg yolk powder, was inadequate in the substances found in yeast or other sources of the vitamin B complex. The lecithin in egg yolk powder, despite its choline content, was probably insufficient in amount, or its lipotropic activity was antagonized by the large amounts of cholesterol and proteins found in egg

Steiner³¹ demonstrated a weight gain in patients with chronic disorders when egg yolk powder was added to the diet, high caloric diets alone failed to give an increase of weight in these patients. His observation is not entirely confirmed in this study on rats, since the feeding of egg yolk powder failed to accentuate weight gain appreciably. Necropsies of the rats fed egg yolk powder revealed a marked increase of fats in all the depots and this should have caused a much greater increase of body weight. It is conceivable that this discrepancy may have been due to fat replacement of muscle fibers

The rats fed egg yolk powder did not disclose an increase in systolic blood pressure. This may be ascribed to the fact that no observable vascular damage occurred in experimental rats. A slight decrease in blood pressure was noted in both control and experimental rats toward the end of the investigation. This may have been due to the adjustment of the rats to handling and environment, or perhaps to myocardial damage produced by the cardiac punctures made at monthly intervals

28 Blatherwick, N. R., Medlar, E. M., Bradshaw, P. J., Post, A. L., and Sawyer, S. D. *Proc Soc Exper Biol & Med* **29** 345, 1931, *J Biol Chem* **97** XXXIII, 1932, **100** LVIII, 1933, **103** 93, 1933

29 Himsworth, H. P., and Glynn, L. E. *Clin Sc* **5** 93, 1944

30 Himsworth, H. P., and Glynn, L. E. *Clin Sc* **5** 133, 1944

31 Steiner and Domanski⁷ Steiner⁹

SUMMARY

The prolonged feeding of egg yolk powder to rats augmented the cholesterol content of the blood and the liver but failed to produce atherosclerosis of the aorta. The lecithin content of the liver was not increased by feeding of egg yolk.

Deposits of fat appeared in the liver six to nine months after the feeding of egg yolk was begun. It follows that under the conditions of this experiment the lecithin present in egg yolk failed to inhibit lipid infiltration of the liver.

During the latter months of the experiment the rats fed egg yolk powder gained weight more rapidly than did the control rats.

The systolic blood pressure was not elevated in rats fed egg yolk powder.

Case Reports

TUMOR OF THE THORACIC INLET PRODUCING THE PANCOAST SYNDROME

A Report of Seventeen Cases and a Review of the Literature

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IN 1838 Hare reported on an illness in a man 40 years of age that began with pain, tingling and numbness along the course of the left ulnar nerve, pain in the left shoulder and swelling of the left elbow, all of one month's duration. Examination disclosed a small tumor in the inferior triangular space of the left side of the neck, a constricted left pupil and a drooping left eyelid. Within three weeks the tumor became larger and the pain more severe. Gradually the lower extremities became numb, cold and then completely paralyzed. Death occurred two and one-half months after the onset of symptoms. Necropsy disclosed edema of the legs and the left arm and a large scirrhous mass in the left side of the neck. The tumor involved the carotid and subclavian arteries and the corresponding veins and the pneumogastric, phrenic and recurrent laryngeal nerves; it pressed on the brachial plexus, it was firmly attached to the spine at the origin of the third and fourth nerves of the plexus, both of which were inseparable from the tumor, and extended into the dura of the spinal cord along the course of the eighth cervical and first dorsal nerves. No carcinoma was demonstrable elsewhere.

In 1850 MacDonnell reported on a similar illness in a girl 17 years old. A tumor gradually developed in the left supraclavicular region, accompanied by pain in the left shoulder and arm, loss of power with atrophy of muscle and edema of the left arm, constriction of the left pupil and ptosis of the left eyelid, cough, dyspnea, partial paralysis, and incontinence of urine and feces. Superficial veins over the tumor became dilated, and pulsation of the left brachial, ulnar and radial arteries was completely obliterated. The patient died in dyspnea. At autopsy an encephaloid tumor of the left lung was found that had extended into the base of the neck, where it was indented by the clavicle and attached to the cervical vertebrae. The tumor surrounded the brachial plexus, pressed on the cervical sympathetic chain, stretched the left subclavian vein and obliterated the third portion of the corresponding artery. It had metastasized to the right lung. In 1918 Ricaldoni (quoted by Romano and Eyherabide) described a carcinoma of the lung occurring in a man 65 years of age, which had invaded the first dorsal vertebra and produced pain in the right shoulder and arm, loss of power and atrophy of muscle of the right arm and hand, and Horner's syndrome on the right side. There were metastases in the cervical lymph nodes. The duration of the illness was five months. In 1921 Freeman reported on a patient 53 years old who, because of pain in the left arm, was thought to have a tumor of the spinal cord.

From the Clinical Laboratories, Jefferson Medical College Hospital

At operation a neoplasm was not found, but subsequent roentgenograms disclosed a tumor at the apex of the left lung that was diagnosed histologically as endothelioma of the pleura

Apparently unaware of the first three of the aforementioned papers, Pancoast in 1924, under the title "Importance of Careful Roentgen Ray Investigations of Apical Chest Tumors," described 3 cases, and again in 1932, under the title "Superior Pulmonary Sulcus Tumors," added 4 others that were essentially similar to the cases already referred to. Although in none of his cases was an autopsy performed and although material was removed for histologic study in only 3 cases, Pancoast expressed the belief that he was dealing with a new entity—a specific tumor arising in an embryonal epithelial rest which was possibly derived from the fifth pharyngeal pouch. As prerequisites for a correct diagnosis of the syndrome he listed the following: location of the thoracic inlet in the region of the superior pulmonary sulcus, pain around the shoulder and down the arm, atrophy of the muscles of the hand, Horner's syndrome and, roentgenographically, a small homogeneous shadow at the extreme apex with more or less local destruction of ribs and infiltration of vertebrae. He expressed the belief that primary tumor of the lung, the pleura, the ribs or the mediastinum could be excluded by the absence of one or more of the foregoing characteristic manifestations. He was, however, careful to state that "it is possible that this new designation may be changed again with a better knowledge of the histopathology of the growth."

Time has borne out the truth of the last statement, for complete necropsies of patients with this syndrome have shown that it is the location of the tumor which is important in producing the chain of symptoms described, and not its histologic structure. It has further been shown that the latter is not uniform but varies from case to case. A search of the medical literature discloses records of 134 cases, including those referred to in the foregoing paragraphs. (A bibliography of the reported cases accompanies this article.)

While in most of these cases the growth has been described as originating in the lungs, almost all other organs have served as primary sites for tumors metastasizing to the thoracic inlet and producing the characteristic clinical manifestations. As will be seen subsequently, however, there are also cases in which a careful autopsy disclosed no primary site other than the soft tissues of the neck, thus confirming Pancoast's original impression that the tumor may arise in an embryonal rest.

It is the purpose of this paper (1) to record 17 cases of tumor of the thoracic inlet which we have encountered at the Jefferson Medical College Hospital in the last six years and which fulfil all or most of the criteria outlined by Pancoast and (2) to analyze the data on all similar cases hitherto recorded of which reports were available to us.

REPORT OF CASES

Primary Carcinoma of the Lung

CASE 1—A white man 39 years old was well until nine weeks before admission, when severe pain developed in the left shoulder, which occasionally radiated down the arm. Gradually there developed weakness, loss of weight, slight cough,

hoarseness, numbness of the legs and difficulty in walking. Examination disclosed shotty nodes in the neck, dullness at the apex of the left lung, Horner's syndrome on the left side, paralysis of the left vocal cord and xanthochromia of the spinal fluid with evidence of block. Bronchoscopy revealed no abnormality. Roentgenograms of the chest showed an infiltration of the upper lobe of the left lung and erosion of the transverse process of the second dorsal vertebra. Gradually paralysis became more severe, and the patient died three and one-half months after the onset of symptoms.

At necropsy there was a primary tumor in the main bronchus to the upper portion of the upper lobe of the left lung that measured 15 cm in length. The bronchial lumen was constricted, and there were several bronchiectatic and abscess cavities distal to the tumor. From the mucosa the neoplasm extended through the bronchial wall and was continuous with a tumor mass 4 cm in diameter that filled the concavity of the aortic arch and contained the left recurrent laryngeal nerve. Attached to this mass were several other tumors, one of which occupied the left thoracic inlet (fig 1). This tumor measured 35 cm in diameter, it completely surrounded the second portion of the subclavian artery and partially encircled and compressed the most inferior bundle of the brachial plexus. Medially it engulfed the inferior portion of the cervical sympathetic trunk, was adherent to the body of the first dorsal vertebra and almost completely replaced that of the second dorsal vertebra. From the latter it extended into the spinal canal, along which it spread for a distance of 4 cm and compressed the corresponding portion of the spinal cord. There were metastases in each kidney and in the abdominal lymph nodes. Microscopically, both the primary tumor and the secondary growths disclosed the usual pleomorphism characteristic of bronchogenic carcinoma. The tumor cells were round or irregular, contained a moderate amount of cytoplasm and were arranged either diffusely or in adenomatous formation. The supporting stroma was dense, fibrous and abundant.

CASE 2—A white man aged 61 was admitted with a history of a lump on the left side of the neck, pain in the left shoulder and down the left arm, and numbness, tingling and coldness of the fingers of the left hand, all of eight weeks' duration. Within two months there developed swelling of the left hand, Horner's syndrome on the left side and pain in the right shoulder. Roentgenograms of the chest first showed only infiltration of the upper lobe of the left lung but later disclosed a tumor in the upper part of the mediastinum and destruction of the lower cervical vertebra. The patient died five months after the onset of symptoms.

Necropsy disclosed a primary tumor 7 mm long in a 4 mm bronchus to the upper lobe of the right lung. The draining nodes were infiltrated with tumor and these were continuous with a mass 6 by 4 cm in the anterior part of the mediastinum. This in turn was continuous with a tumor in the left thoracic inlet that measured 8 cm in diameter. The neoplasm partially encircled the left subclavian artery and completely surrounded the lower trunks of the brachial plexus and the left recurrent laryngeal nerve. It eroded the posterior portion of the left rib and the eighth cervical vertebra. The left subclavian vein was thrombosed. There were metastases in the lower lobe of the left lung and the liver. Microscopically both the primary tumor and the metastatic growths disclosed anaplastic carcinoma of the "oat cell" variety in which there was a scanty stroma of connective tissue.

CASE 3—A white man 65 years old began to have pain in the right side of the face, the shoulder and the arm three months before admission. At the time of entry there were weakness of the right arm, dyspnea, cough with hemoptysis, dysphagia, hoarseness, paralysis of the right vocal cord, constriction of the pupil

and a large mass over the right mandible. Roentgenograms disclosed destruction of the right side of the mandible and "old tuberculosis" at the apex of the right lung. The patient died four months after the onset of pain.

Necropsy disclosed a primary tumor of the right main bronchus that measured 2 cm in length. It penetrated the bronchial wall and produced an extrabronchial mass of firm gray tissue 4 cm in diameter. This was continuous with a series of smaller masses in the superior part of the mediastinum, which were attached to a



Fig 1 (case 1) —Anterior view of the tumor in the left thoracic inlet, where it engulfed some of the structures and compressed others. (a) cervical sympathetic trunk, (b) tumor, (c) brachial plexus, (d) subclavian artery, (e) subclavian artery.

tumor in the right thoracic inlet that measured 8 cm in diameter. This neoplasm encircled the subclavian and common carotid arteries, the vagus and recurrent laryngeal nerves, and the inferior portion of the cervical sympathetic trunk. The upper branches of the brachial plexus passed over the tumor, while the lower

two roots were partially surrounded by the neoplastic tissue. Most of the first rib was destroyed by the tumor, and there were metastases in each kidney. Microscopically, both the primary tumor and the secondary growths were predominantly of a squamous cell variety.

CASE 4—A white man 60 years old gave a history of progressive dyspnea and loss of 40 pounds (18 Kg) in weight in the last five months. More recently he had suffered from repeated colds and a cough. He had pain down both arms, weakness of the right arm, and Horner's syndrome on the right side. One day after admission flaccid paralysis of the right arm and leg developed. He died on the following day.

Necropsy disclosed a primary tumor of the left main bronchus that measured 25 cm in length. It penetrated the bronchial wall and infiltrated the mediastinum with a firm, solid, pink to gray mass 5 cm in diameter. This was continuous with another mass, 14 by 6 cm, in the upper part of the mediastinum and the right thoracic inlet. The latter occupied the concavity of the first rib and protruded into the neck for a distance of 5 cm. It surrounded the first portion of the subclavian artery, the vagus and recurrent laryngeal nerves and the cervical sympathetic plexus. The lower roots of the brachial plexus as they left the foramina were pressed on but were not encircled by the tumor. There were metastases in the liver and the abdominal nodes. Microscopically, the tumor was of an anaplastic "oat cell" variety with some attempt at adenomatous formation.

CASE 5—A 45 year old white man was admitted with the following complaints: pain in the left scapula of two years' duration, which was diagnosed as neuritis; cough with hemoptysis, of three months' duration; loss of weight; weakness of the left arm of six weeks' duration. He had Horner's syndrome on the left side, slight hoarseness, dulness at the apex of the left lung and a shadow in the same region roentgenographically. He died three days after admission.

Necropsy disclosed a primary tumor in the left main bronchus 6 cm beyond its origin, which had infiltrated the adjoining lung, was adherent to the first rib and occupied the region of the superior pulmonary sulcus. There were metastases in the kidneys, the adrenal glands, the mesenteric lymph nodes, the liver and the brain. Microscopically, the tumor was of a squamous and anaplastic cell variety and contained an abundant fibrous tissue stroma.

CASE 6—A white man 48 years old strained his back two and a half months before admission. This was followed by pain that gradually increased in intensity. Four weeks later there were tingling and numbness in the lower extremities, with inability to walk and incontinence of urine and feces. At the time of entry there were dyspnea, Horner's syndrome on the right side, tumor in the right side of the neck and flaccid paralysis of the lower extremities. Roentgenograms showed a normal spine and bronchogenic carcinoma of the upper lobe of the right lung. The patient died one week after admission.

Necropsy showed the main bronchus to the upper lobe of the right lung occluded by a primary tumor 2 cm long. It infiltrated the lung for a distance of 2 cm and was continuous with a firm, gray mediastinal tumor 7 cm in diameter. The upper portion of this projected into the right thoracic inlet, where it partially encircled the right subclavian artery and the recurrent laryngeal nerve and was adjacent to the inferior portion of the brachial plexus. Tumor tissue replaced almost all the vertebrae and from these extended directly into the spinal canal in six places. There were metastases in the pancreas, the liver, the colon, the lymph nodes and the testes. Histologically, the tumor was of an anaplastic cell variety.

CASE 7—A white man 46 years old was admitted with pain in the right shoulder of two months' duration. Roentgenograms of the chest showed a tumor of the upper lobe of the right lung. At thoracotomy an inoperable cancer was found, and biopsy revealed squamous and adenocarcinoma. In spite of roentgen therapy the patient lost weight and became dyspneic. When he returned nineteen months later, he had Horner's syndrome on the right side, severe edema of the right side of the face, the neck and the upper extremity, pain in the right shoulder and down the right arm, a tumor in the left supraclavicular fossa and, roentgenographically, a tumor of the upper lobe of the right lung with destruction of the ribs and vertebrae. He died twenty-one months after the onset of pain. There was no autopsy.

CASE 8—A white man aged 56 was admitted with continuous pain of the left side of the chest and the shoulder of eighteen months' duration. Gradually the pain extended down the left arm, and there developed cough, hoarseness, paralysis of the left vocal cord, dyspnea, Horner's syndrome on the left side, dulness and, roentgenographically, increased density of the apex of the left lung, and loss of weight. Thoracotomy disclosed irremovable carcinoma of the left lung. Biopsy of the tumor revealed anaplastic epithelial cells embedded in a dense fibrous tissue stroma. The patient was discharged from the hospital and there was no further follow-up.

CASE 9—A white man 45 years old had pain in the anterior portion of the chest that radiated to the back and down the left arm of three months' duration. It was diagnosed as neuritis and treated with thirteen "electric treatments." Gradually there developed cough, loss of weight, and drooping of the left eyelid. Examination disclosed Horner's syndrome on the left side, dulness at the apex of the left lung and, by roentgenogram, a neoplastic infiltration of the upper lobe of the left lung. At operation an irremovable carcinoma of the left lung was encountered. Biopsy of the tumor disclosed large sheets of epithelial cells supported by a scanty fibrous tissue stroma, interpreted as compatible with a primary carcinoma of the lung. The patient was returned to the family physician for roentgen therapy.

Primary Bronchial Rest Carcinoma

CASE 10—A white man 57 years old complained of neuritis of the arm for eight months and cough and hoarseness for three weeks. Examination disclosed some swelling in the right supraclavicular fossa, and roentgenographically there was haziness of the medial border of the right upper lung field. Gradually the pain extended to the right side of the neck and the face and down the arm and became unbearable. There was paralysis of the right vocal cord, Horner's syndrome on the right side, loss of weight, dyspnea, dysphagia necessitating gastrostomy, edema of the neck, the shoulder and the arm, an increase in size of the supraclavicular tumor and, roentgenographically, partial destruction of the first right rib. He died eighteen months after the onset of pain.

Necropsy disclosed Horner's syndrome on the right side, edema of the entire upper extremity and a tumor in the right supraclavicular fossa. The latter was dense and sclerotic, and measured about 15 cm in greatest diameter. It filled the entire right thoracic inlet, partially destroyed the manubrium, the clavicle and the inner portion of the first rib and was adherent to, but did not infiltrate, the vertebral bodies. It completely surrounded the subclavian artery and vein, the carotid vessels, the sympathetic plexus, the vagus nerve and the entire brachial plexus (fig 2). Medially it pushed the esophagus and the trachea to the left, partly infiltrated these and extended over the trachea to the left side. The thyroid

gland was carefully examined. The left lobe was untouched, but the inferior pole of the right lobe was enveloped by the tumor. Everywhere, however, it was separated from the neoplasm by edematous connective tissue, and its capsule was intact. The visceral pleura of the apex of the upper lobe of the right lung was adherent to the tumor, but the lung was not invaded. The subclavian vessels were completely thrombosed. No tumors could be found in any other organs of the body, including the prostate, the mouth and the nasopharynx.

Microscopically, all sections disclosed an abundance of very dense, acellular fibrous tissue stroma containing cancer cells in anaplastic, adenomatous and



Fig 2 (case 10)—Anterior view of a large scirrhous tumor of the right thoracic inlet, where it surrounded nerves and vessels. Note that the thyroid gland and the right lung are not a part of the tumor. (a) sympathetic plexus and vagus nerve, (b) brachial plexus, (c) axillary artery and vein, (d) clavicular groove, (e) tumor, (f) common carotid artery, (g) thyroid gland, (h) innominate artery, (i) lung.

squamous cell formation (fig 3). The anaplastic cells were relatively small and round, oval or irregular, had abundant pink-staining cytoplasm and small, round or irregular, deeply stained nuclei. The cells in glandular formation were cuboidal, with abundant, less densely stained pink cytoplasm and round or oval vesicular nuclei in a basilar position. The cells arranged in squamous formation disclosed

indistinct borders, lightly stained pink cytoplasm and round or oval vesicular nuclei. Occasionally the masses contained hyaline bodies, but there were no definite pearls or intercellular bridges. Numerous sections were taken through the junction of the right lobe of the thyroid gland and the tumor, and in all the sections the latter was sharply demarcated from the former. Many sections from each lobe of the



Fig 3 (case 10) — *A*, section from the tumor mass showing a background of dense fibrous tissue containing anaplastic cancer cells. *B*, section from another portion of the tumor showing cancer cells in glandular formation. *C*, section from still another portion of the tumor showing the cancer in squamous cell formation. Hematoxylin and eosin. $\times 200$

thyroid gland likewise disclosed no cancer cells. They were, however, found within blood vessels in the tumor proper, in the medulla of one adrenal gland and in the interstitial tissue of one testis.

CASE 11—A white man 52 years of age began to have pain in the left scapula and shoulder nine months before admission, which was diagnosed as neuritis. Gradually it became more severe and radiated down the inner aspect of the left arm. There developed a dry cough, dyspnea, paralysis of the left arm and both lower extremities and incontinence of urine and feces. Examination disclosed a mass in the left supraclavicular fossa, Horner's syndrome on the left side, edema of the left arm and hand and, roentgenographically, a mass in the neck encroaching on the apex of the left lung and partly destroying the first rib. He died one day after admission.

Necropsy disclosed a neoplasm in the left thoracic inlet that measured 8 cm in diameter. It was very firm, gray to white, surrounded all the vessels and nerves in this region, was attached to the visceral pleura of the apex of the upper lobe of the left lung but did not penetrate the lung tissue, eroded the posterior portions of the first two ribs and the bodies of the corresponding vertebrae, and penetrated directly into the spinal canal, where it compressed the cord for a distance of 4 cm. Careful examination of all organs, including the thyroid gland and the prostate, disclosed no other tumors. Histologically the cancer was identical with that of case 10. There was a dense fibrous tissue stroma in which the neoplastic cells were in anaplastic, adenomatous and squamous cell formation. Only the pleura at the apex of the upper lobe of the left lung was involved with tumor. Sections of the thyroid gland and of the prostate disclosed no primary or secondary carcinoma, and there were no metastases to any of the other organs.

CASE 12—A 39 year old white man gave a history of pain in the back and the right shoulder, dyspnea, cough and loss of weight, all over a period of eight months. Examination disclosed loss of power of the right arm, absence of Horner's syndrome and, roentgenographically, a mass in the apex of the right lung with destruction of the second and third ribs and of the bodies of the corresponding vertebrae. He died nine months after the onset of pain.

Necropsy disclosed a tumor occupying the right thoracic inlet and the base of the right side of the neck, measuring 8 cm in diameter. It was firm, gray, and protruded into the thoracic cavity for 2 cm. It eroded portions of the first three ribs and the bodies of the corresponding vertebrae and compressed the adjacent spinal cord. The apex of the lung was infiltrated with tumor for a distance of 1 cm, and there were metastases in the kidneys. Histologically, as in cases 10 and 11, there was a background of dense fibrous tissue in which epithelial cells were embedded in anaplastic, adenomatous and squamous cell formation. In this case, however, the third type containing definite pearls predominated and the stroma was less abundant. There were metastases in the kidneys and in a cervical lymph node but not in any of the other organs.

Primary Carcinoma of the Thyroid Gland

CASE 13—A white woman aged 69 gave a history of nervousness, dysphagia and loss of weight of seven months' duration, and pain in the left side of the neck and down the left arm, with weakness of the left arm, of four months' duration. There were hoarseness, Horner's syndrome on the left side, a greatly enlarged, hard, tender thyroid gland and atrophy of the muscles of the left arm, followed by edema. A roentgenogram showed a tumor mass in the lower part of the neck, the mediastinum and the left thoracic inlet. The patient died eight months after the onset of symptoms.

Necropsy disclosed a scirrhous gray tumor replacing the entire left lobe of the thyroid gland and the lower three quarters of the right lobe and measuring 15 by 12 by 6 cm (fig 4). Inferiorly it extended beneath the sternum into the

anterior mediastinum, where it compressed the trachea to a narrow slit. Laterally it completely encircled the left subclavian and carotid vessels, the lower portion of the cervical sympathetic trunk, the left recurrent laryngeal nerve and the lower roots of the brachial plexus. The tumor did not destroy the ribs or the vertebrae, nor did it enter the spinal canal. There were metastases in the lungs, the heart, the left adrenal gland, the liver, the kidneys, the mucosa of the small bowel, the abdominal lymph nodes and the subcutaneous tissue of the back.



Fig 4 (case 13)—Anterior view of a primary carcinoma of the thyroid gland engulfing all the vessels and nerves in the vicinity. (a) sympathetic nerve, (b) brachial plexus, (c) subclavian artery, (d) thyroid cartilage, (e) vagus nerve and common carotid artery, (f) tumor.

Histologically, most of the primary and secondary tumors were composed of round or spindle cells with a moderate amount of cytoplasm and round or oval, evenly stained nuclei arranged in a sarcomatous manner. There were other areas, however, that consisted of cuboidal cells in adenomatous and papillary formation, and still others composed of huge bizarre cells with abundant irregular cytoplasm and possessing equally huge and bizarre hyperchromatic nuclei.

CASE 14—A Negro aged 58 stated that he noticed a change in his voice eight weeks previously. Soon there developed pain and swelling in the neck. Examination disclosed a large mass in the right anterolateral portion of the neck that measured 10 cm in diameter. There were hoarseness, laryngeal paralysis, dyspnea, cough and dysphagia, and a roentgenogram showed a large mass in the neck pushing the trachea to the left. Dyspnea became so severe that tracheotomy was performed, at which time the tumor was observed to cover the trachea in the region of the thyroid gland, necessitating removal of a portion of it in order to produce an airway. The patient died about three months after the onset of symptoms. There was no autopsy, but histologic sections of tissue removed at operation disclosed a cancer very similar to that in case 13. Most of it was composed of round or spindle cells, producing a sarcomatous appearance, but there were also some polyhedral cells in an anaplastic distribution and others in an adenomatous formation.

Primary Mesothelioma of the Pleura

CASE 15—A white man 41 years old complained of pain and numbness of his left arm and loss of 45 pounds (20.5 Kg) over a period of eight months. He also noticed "floods" of heat over the left side of his face and neck with absence of perspiration. Horner's syndrome was present on the left side, and there were dyspnea, dysphagia, cough, atrophy of muscle and loss of power in the left arm, fulness in the left supraclavicular fossa, dullness over the apex of the left lung and, roentgenologically, a tumor in the same region with destruction of the first rib. He died nine months after the onset of pain.

Necropsy disclosed a hard, gray, flat pleural and subpleural tumor covering the apex of the left thoracic cage. It measured 25 cm in thickness and 14 cm in diameter. It extended to the left and upper portion of the mediastinum and the pericardium, partly surrounded the subclavian artery, completely engulfed that portion of the brachial plexus posterior to this vessel together with the lower portion of the sympathetic plexus, and partly eroded the first two ribs. The upper part of the brachial plexus was not involved. The tumor extended to the pericardium, and there was a metastasis in the right crus of the diaphragm and in the adjoining lymph nodes, adrenal gland and kidney. The remaining organs, including the thyroid gland and the prostate, were normal. Histologically there was an abundant stroma of dense, acellular fibrous tissue in which there were groups of cancer cells. Some groups were solid and composed of irregular cells with abundant watery cytoplasm and irregular deep-staining nuclei, whereas others were in adenomatous formation wherein the cells, although similar, were cuboidal and the nuclei basilar in position. There were no tumors in either the thyroid gland or the prostate, or in any of the other organs not already referred to.

Primary Carcinoma of the Larynx

CASE 16—A white man aged 69 was admitted because of cough and pain in the right ear of several months' duration and hoarseness of three months' duration. Examination disclosed a firm right lobe of the thyroid gland and a primary cancer of the right vocal cord and the right side of the larynx. This was confirmed both

by biopsy and examination of the entire specimen after laryngectomy, when the growth proved to be a squamous cell carcinoma. Examination of the patient four months later revealed a fixed nodule above and to the right of the tracheal stoma and tenderness along the course of the great vessels. An attempt was made to extirpate the mass but, owing to the extensive involvement, all the tumor could not be removed. Histologic examination of removed tissue disclosed a squamous cell carcinoma. Nine months later the patient returned with severe pain in the right shoulder and down the right arm, and some dyspnea. Examination disclosed a large supraclavicular mass on the right side, "fixed to the brachial plexus," and Horner's syndrome of the right side. The patient was discharged from the hospital unrelieved of his pain and is still living.

Primary Hodgkin's Disease

CASE 17—A white man 21 years old was admitted with a history of a dry cough of two months' duration and swelling of the right side of the neck of three weeks' duration. There were enlarged lymph nodes in the right side of the neck and in the groins, and roentgen examination disclosed a mass in the superior mediastinum. Biopsy of one of the nodes revealed Hodgkin's disease, for which he was given roentgen therapy. He returned ten months later with dull pain in the left shoulder that radiated to the elbow. Horner's syndrome was noted on the left side, with dulness at the apex of the left lung, and roentgen examination showed a tumor in the superior mediastinum and the left thoracic inlet without apparent involvement of bone. He died fourteen months after the onset of symptoms.

Necropsy disclosed edema of the left side of the face and neck. In the upper and anterior mediastinum there was a gray firm nodular mass that measured 8 by 8 by 5 cm. It surrounded the main branches of the aorta and the left innominate vein and extended directly into the base and the left side of the neck and the left thoracic inlet, where it surrounded most of the structures. There was tumor involvement of the pleura, the diaphragm, the lymph nodes, the left sixth rib and the left kidney. Histologically the lesion was a typical one of Hodgkin's disease in which there was extensive fibrosis.

ANALYSIS OF REPORTED CASES

Nomenclature—Although prior to 1924 there were isolated reports of tumor in the thoracic inlet producing the train of symptoms already referred to, it was not until Pancoast's report that such a syndrome became generally known. In his first paper Pancoast spoke of the lesion as "apical chest tumor" but in 1932 he changed the title to "superior pulmonary sulcus tumor" under the assumption that he was dealing with a specific tumor. While most subsequent authors used the latter title, the syndrome has been described under a variety of names. Some of the more commonly employed have been "apico-costovertebral syndrome" (Tobias and other Latin American authors), "cancer of the thoracic pulmonary apex" (Pardal and Brea), "superior pulmonary sulcus syndrome" (Gilt and Trawbridge), "sulcus tumor" (Breslin), "primary apical lung carcinoma" (Steiner and Francis), "extrapulmonary tumors of the thorax" (Pierce), "sterno-clavicular branchiomas" (Fried), "tumor of the superior thoracic inlet" (Kelman and Schlezinger) and "Pancoast syndrome" (Fernandez and Fuste). Many other names, referring to specific tumors, have been used. Obviously, since any neoplasm, regardless of its origin, can produce

the signs and symptoms indicated, titles referring to a particular tumor or tumors should be avoided. Although in all our cases in which autopsy was performed, the subclavian artery was involved with tumor and therefore the neoplasm was in reality in the superior pulmonary sulcus, we believe that such a designation is too limited, for in each case the tumor also involved the entire thoracic inlet and even the base of the neck. We therefore believe that the neoplasms should be spoken of as tumor of the thoracic inlet. Also, since it was Pancoast who first emphasized the combination of tumor in the apex of the chest with more or less destruction of adjacent ribs and vertebrae, Horner's syndrome and pain down the arm, we believe that the simplest means of expressing this is by designating it as the Pancoast syndrome. For these reasons it is our opinion that the most suitable and comprehensive title is "Tumor of the Thoracic Inlet Producing the Pancoast Syndrome."

Clinical Considerations—From the literature there were available to us reports on 134 cases, which together with the 17 cases included in this report make a total of 151 cases. In addition to these there are mentioned, particularly in discussions of papers read at meetings, 31 other cases. Since data on these are incomplete they will be omitted from discussion except when their inclusion is specifically mentioned.

Of the 151 patients, 137 were male and 14 female. The ages were 16 to 73 years, with an average of 48.6 years. The recorded duration of the illness from the initial symptoms to death in 113 cases averaged 10.5 months and ranged from two to thirty-two months. The right side was involved in 85 patients, the left side, in 66. A tumor mass was disclosed in the thoracic apex roentgenographically in 140 cases. The frequency of occurrence of the more important clinical manifestations was as follows: destruction of ribs, 72; destruction of vertebrae, 41; Horner's syndrome, 133; pain in the shoulder, 129; and down the arm, 122; loss of power in the arm, 99; dulness on percussion at the apex of the lung, 82; palpable supraclavicular tumor mass, 74, of which 58 were also visible; atrophy of the muscles of the arm, 71; cough, 48; dyspnea, 30; hoarseness, 22; edema of the arm, 20; laryngeal paralysis, 15; and hemoptysis, 6.

As is apparent from the accompanying photographs, all clinical manifestations in the syndrome can be accounted for on the basis of tumor involvement of the nerves and the blood vessels. The diagnosis is made from a consideration of the signs and symptoms, the roentgenograms of the apex of the chest and of the neck, and the results of biopsy of the tumor. When, however, the results of roentgen and histologic study are positive it is already too late. To date the disease has been 100 per cent fatal. Roentgen therapy so far appears to be of no avail, and certainly when the neoplasm is demonstrable by either physical or roentgen examination, one can be sure that the vital structures are involved to such an extent that complete surgical removal is impossible. Frequently, however, the patient has consulted a physician when pain in the shoulder and arm first developed, and because an obvious cause could not be found the condition has been diagnosed for months as neuritis. Obviously, if the tumor is metastatic, nothing in the way of curing it can be done even at an early stage, but if the lesion is primary in the neck, there may be a faint chance of complete extirpa-

tion when the tumor produces the first symptoms. It appears, therefore, that the cause of any persistent neuritis in the shoulder or the arm should be thoroughly investigated, even by surgical exploration if necessary.

Pathologic Considerations—Autopsy was performed in 75 of the 151 cases, and biopsy alone in 29 others, making a total of 104 cases in which the tumor was studied histologically. There were 2 intraspinal lesions and 149 extraspinal neoplasms. The former consisted of syringomyelic cyst of the cord and leptomeningitis. The distribution of the primary cancers in the latter group were carcinoma of the lung, 100, primary carcinoma of the neck, 21, endothelioma of the pleura, 5, carcinoma of the thyroid gland, 4, carcinoma of the breast, 3, carcinoma of the esophagus, 2, sympathoblastoma, 2, round cell sarcoma, hypernephroma, thymoma, mediastinal tumor, osteogenic sarcoma, carcinoma of the pancreas, neurogenic fibrosarcoma, chondrosarcoma, carcinoma of the stomach, carcinoma of the prostate, carcinoma of the larynx and Hodgkin's disease, 1 each. In the 32 cases mentioned but not described the distribution was carcinoma of the lung, 10, sarcoma, 2, carcinoma of the breast, thymoma, carcinoma of the stomach, endothelioma of the pleura, sympathicoblastoma and Hodgkin's disease, 1 each. In the reports of 14 other cases the primary sites were not stated.

Of particular interest, perhaps, are those cases in which the tumor was said to have originated in the neck. In none of the cases without a complete and thorough autopsy can the tumor be designated as arising in the neck, and even if such an examination was made, one is hesitant in saying that some primary focus elsewhere was not missed. Thus, among the 18 cases in the literature that were thought to be instances of tumor of branchiogenic origin, there were only 5 (3 reported by Fried, Graf and Sternberg, and Clarke, respectively, and 2 by Morris and Harken) in which the tumor was examined completely enough to be accepted as possibly primary in the neck. Of these 5 cases, 4 were instances of squamous cell carcinoma and 1 was an instance of squamous cell and adenocarcinoma. To these may be added 3 of our cases (10, 11 and 12) in each of which the cells were in anaplastic, squamous and adenomatous formation.

If in these cases the tumor of the thoracic inlet was primary in the neck, then what structure was responsible for such a varied histologic picture? Most authors who have maintained that such a tumor may originate in the cervical region have expressed the belief that it arises from a branchiogenic rest (particularly the fifth), and Fried has even called it sternoclavicular branchioma. While this may be the explanation for those cases in which the tumor shows a pure squamous cell arrangement it can hardly account for those in which it shows in addition cells in anaplastic and adenomatous formations, because the branchial cyst is always lined with stratified squamous epithelium, and cancer developing from it is always of a squamous cell variety (Carp and Stout, Crile and Kearns). An origin from aberrant thyroid tissue cannot be easily dismissed except for the fact that although carcinoma of the thyroid gland is notoriously pleomorphic, squamous cells ordinarily are not one of the varied types of cells encountered. An explanation which has not been previously advanced and which to us seems most

plausible is that the tumor takes origin from a bronchogenic rest. The literature contains reports of 35 cases of bronchial cyst (Laipply). This probably arises as a pinching off of a portion of the foregut at the time of development of the trachea. It is located at some point along the course of the tracheobronchial tree and is lined with ciliated epithelium identical with that lining the trachea and the bronchi. Although no epithelium-lined cyst has been described in cases of primary carcinoma of the thoracic inlet, there is no reason why there may not be misplaced solid groups of cells arising in a similar manner from which such a neoplasm can arise. The location is correct for an origin from such a rest, and certainly the varied histologic picture presented in our 3 cases is highly reminiscent of ordinary bronchogenic carcinoma. This is to be expected since both the bronchial cyst and the bronchi and the trachea are lined with similar epithelium.

SUMMARY

A review of the literature discloses reports of 134 cases of tumor occurring in the thoracic inlet and producing the Pancoast syndrome. To these are added 17 cases, making a total of 151 cases. In the majority the tumor was secondary to a primary focus located in some organ of the body, with primary carcinoma of the lung predominating, although most organs have been named as the site of the primary growth. In 21 cases the tumor was thought to be primary in the neck but in only 8 of these was the examination thorough enough to exclude with reasonable certainty an origin in some distant organ. Because in some of these cases cancer cells were observed in squamous, anaplastic and adenomatous formations, it is believed that they may arise in bronchial rather than branchial rests.

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STEM CELL LYMPHOMA OF THE NEWBORN

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IN THE literature are many reports of various types of congenital cancer, but only a few contain unequivocal data revealing both the congenital and the cancerous nature of the disease. It is my purpose to discuss here the surprisingly high incidence of congenital cancers, and to report a case of congenital lymphoma of the stem cell type. To my knowledge there is no record of a similar case in the literature.

In view of Wells's¹ comprehensive critical analysis of the literature concerning congenital cancers, published in 1940, it would be superfluous to review the literature again at this time. Wells has classified all the reported cases as "accepted, probable or possible" cases, depending on the data given. He has summarized the material as shown in the accompanying table.

Summary of Recorded Congenitally Malignant Tumors (Not Including Retinal Tumors) (Quoted from Wells¹)

| | Accepted | Probable | Possible | Total |
|--|----------|----------|----------|-------|
| Malignant renal tumors | 5 | 11 | ? | 16 |
| Malignant adrenal neuroblastoma | 17 | 15 | 21 | 53 |
| Malignant extra adrenal neuroblastoma | 6 | 4 | ? | 10 |
| Congenital sarcoma | 33 | 29 | 53 | 115 |
| Teratoma, malignant at birth | 1 | 2 | ? | 3 |
| Tumors of undetermined nature | 0 | 1 | 7 | 11 |
| Carcinoma of liver | 0 | 1 | 9 | 10 |
| Malignant hemangioendothelioma of liver | ? | ? | 15 | 15 |
| Hepatic tumors of undetermined character | ? | ? | 4 | 4 |
| Carcinoma, excluding liver | 0 | 0 | 5 | 5 |
| Cerebral glioma | 1 | 1 | 2 | 4 |
| Malignant endothelioma, excluding liver | ? | ? | 5 | 5 |
| Melanoma malignum | 0 | 2 | 2 | 4 |
| | 60 | 66 | 123 | 255 |

It will be noted in the table that there is no accepted case of congenital carcinoma. Recently Ziegler² reported bilateral ovarian carcinoma occurring in a 30 week old fetus. Congenital sarcoma, on the other hand, comprises the largest individual group of neoplasms, accounting for approximately half the total number of reports. It is to be remem-

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1 Wells, H. G. Arch Path 30 535, 1940

2 Ziegler, E. E. Arch Path 40 279, 1945

bered that the table deals with congenital cancers and that those listed differ from the types of cancer usually observed in infants, namely, (1) adenosarcoma, (2) retinoblastoma and (3) cancerous neurocytoma of the adrenal gland³. Whereas sarcoma occurred in approximately 50 per cent of the aforementioned highly selected cases,¹ no instance of a cancerous lymphoma is recorded. It is entirely likely, however, that cases of this type are submerged in the large group classified under the general heading "congenital sarcoma". Since Wells's comprehensive review in 1940, Rigby and Holinger⁴ have reported the case of a 17 day old white boy with a mass in the larynx which proved on microscopic examination to be fibrosarcoma. The congenital nature of this tumor is open to question since it was first noted many days after birth.

A review of the literature under the general heading "congenital cancers" thus fails to establish an authentic instance of a congenitally cancerous lymphoma. Likewise, a survey of reports on cancerous lymphoma does not reveal any reported case of this disorder occurring at birth. Baldrige and Awie⁵ reported 20 cases of lymphoma occurring in the first decade of life but gave no further analysis of the age incidence. The youngest patient in the 196 cases of lymphosarcoma reviewed by Sugarbaker and Craver⁶ was a 4 year old child. The age incidence of the 545 cases of lymphoma reported by Gall and Mallory⁷ is again given in decades, with 24 per cent of the cases of Hodgkin's disease, 18 per cent of the cases of lymphoblastic lymphoma and 11 per cent of the cases of lymphocytic lymphoma falling in the first two decades of life. The clasmatic lymphoma, the follicular lymphoma and, to a lesser degree, the stem cell lymphoma occurred predominantly in the older age group. In a personal communication Gall stated that in this series there was 1 case of congenital lymphoblastic lymphoma with leukemia. Jackson and Parker⁸ expressed the opinion that it is uncommon for Hodgkin's disease to appear during infancy and extreme old age, but they admit that it may occur at any age. Smith⁹ in 1934 added 23 cases of Hodgkin's disease of childhood to the 105 cases already noted in the literature. In 6 of the 105 cases the onset of the disease occurred during infancy—the infants ranging from 2 to 4 months of age. It is interesting to note that in 107 of the entire group of 128 cases the disease occurred in boys, being comparable in respect to the sex preference to leukemia and lymphoma. This superficial resemblance

3 Morehead, R. T. *Arch Path* **38** 141, 1944

4 Rigby, R. G., and Holinger, P. H. *Arch Otolaryng* **37** 425, 1943

5 Baldrige, C. W., and Awie, C. D. *Arch Int Med* **45** 161, 1930

6 Sugarbaker, E. D., and Craver, L. F. *J A M A* **115** 17 and 112, 1940.

7 Gall, E. A., and Mallory, T. B. *Am J Path* **18** 381, 1942

8 Jackson, H., and Parker, F. *New England J Med* **230** 1, 1944

9 Smith, C. A. *J Pediat* **4** 12, 1934

is carried still further by Herbut, Miller and Erf,¹⁰ who expressed the belief that Hodgkin's disease, lymphosarcoma and reticulum cell sarcoma arise from a common parent cell—the reticulum cell

REPORT OF A CASE

A full term girl weighing 7 pounds, 8 ounces (3,402 Gm) was born at New Britain General Hospital, Feb 25, 1945, and at the time of birth had on the chest, over the manubrium, an irregular, smooth, salmon pink, waxy tumor. This measured 3 by 2 by 0.2 cm, being intimately associated with the skin and moving with the skin over the subcutaneous tissue. There was also a small palpable lymph node in the left axilla. The remainder of the immediate postnatal examination showed no abnormality.

The initial blood count was 4,800,000 erythrocytes, the hemoglobin content was 15.6 Gm, the leukocyte count was 6,500, with neutrophils 65, eosinophils 3, lymphocytes 24 and monocytes 8 per cent. The patient's blood was type A and in addition her erythrocytes were Rh negative. The Rh types of the parents' erythrocytes are not known. Aside from a moderate reduction of the red cell count and of the hemoglobin content (controlled with multiple transfusions) repeated hemograms at a later date varied little from the foregoing picture. The initial urinalysis gave negative results. A biopsy of the subcutaneous tumor March 1 revealed leukemia cutis, and a biopsy of the enlarged gland of the left axilla March 7 revealed evidence of cancerous lymphoma. A roentgenogram of the chest March 8 showed nothing abnormal.

Roentgen therapy was started March 15, with good immediate results. The large tumor regressed but did not disappear. A few weeks later other cutaneous and subcutaneous masses appeared and were controlled by high voltage roentgen therapy. The patient was discharged to the tumor clinic April 19, weighing 8 pounds, 2 ounces (3,685 Gm). While she was being followed in the tumor clinic, roentgen therapy was continued, but her general condition gradually and progressively declined. Numerous cutaneous and subcutaneous tumor masses appeared over many areas of the body.

The patient was readmitted to the New Britain General Hospital June 21, primarily because of difficulty in breathing. Examination revealed a dyspneic, malnourished and maldeveloped 5 month old girl weighing 10 pounds, 9 ounces (4,791 Gm) with numerous small and large cutaneous and subcutaneous tumor nodules over her entire body. The right side of the chest was flat to percussion, and the breath sounds were markedly diminished. The left side of the chest showed compensatory emphysema.

The hemogram on this admission revealed 3,000,000 erythrocytes, 10.5 Gm of hemoglobin and 6,800 leukocytes, with 53 per cent polymorphonuclear cells, 37 per cent lymphocytes and 10 per cent monocytes. The white cell count later rose to 15,000 with an essentially similar differential count. A roentgen metastatic series showed enlarged hilar glands, hydrothorax on the right, metastatic tumor of the right lung and pleura, metastatic tumor of the left ileum, mediastinal hernia and an enlarged liver.

June 25 and 26, 150 cc and 200 cc, respectively, of straw-colored fluid were withdrawn from the right side of the chest. This fluid contained large mononuclear cells suggestive of a neoplastic origin. The infant gradually became

weaker and began to refuse her feedings. She was given multiple transfusions and symptomatic therapy. She died August 7.

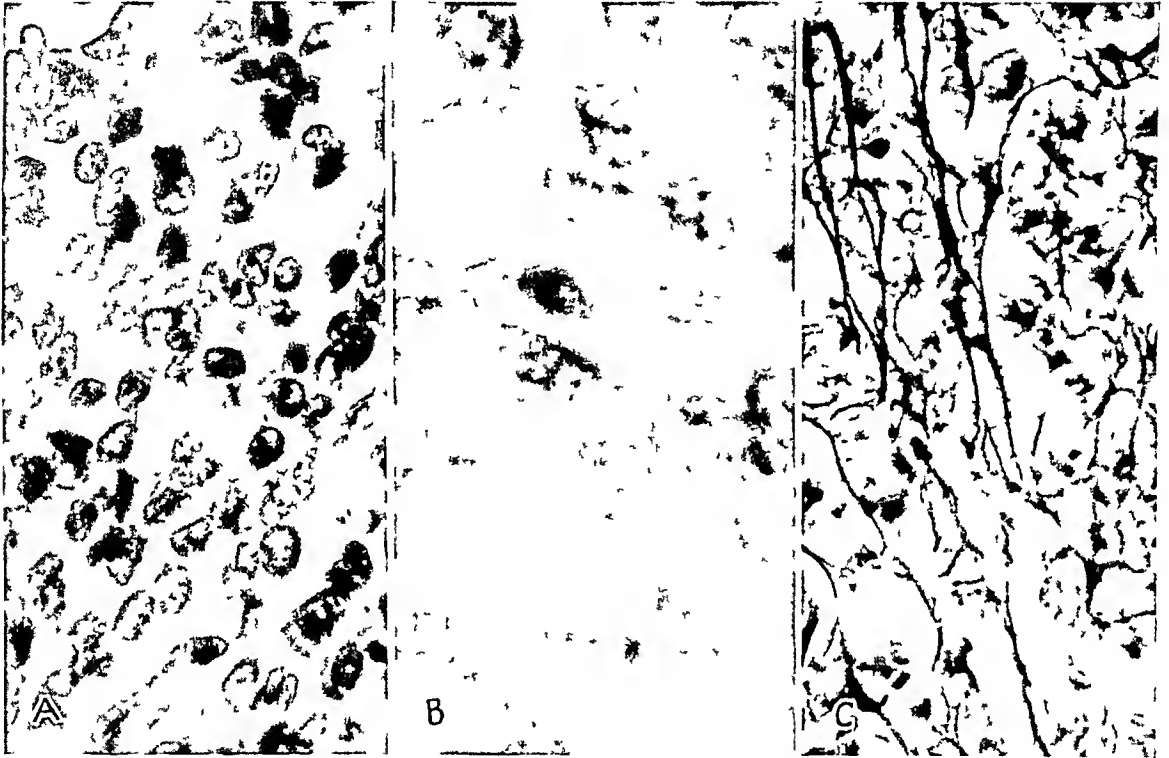
Autopsy—The body was that of a 5 month old white girl weighing 10 pounds 8 ounces (4,762.5 Gm) and measuring 63 cm from vertex to heel. There were generalized pallor and moderate lividity of the thorax, the left side of the face, the skull and the dorsum of the trunk. Rigor mortis was not present. The child was greatly emaciated and cachectic. The striking objective finding was the presence of numerous cutaneous and subcutaneous nodules over the entire body. The distribution of the larger masses coincided in the main with that of lymph nodes. The cutaneous nodes were in general the smaller ones and were clearly demarcated from the surrounding tissue. They were firm, smooth and yellow, had an approximate diameter of 1 cm and were elevated approximately 0.2 cm above the surface of the skin. The larger nodes were not attached to the skin, nor did they cause any discoloration of it, they were only slightly movable, were moderately firm and were composed of many matted enlarged glands. The main distribution of these matted large glands was confined to the axillary and the inguinal areas. However, both large and small masses of tumor tissue were present over the left side of the head, the left temporal area, the right cheek, both axillas, both arms and forearms, the abdomen, the chest, the dorsum of the trunk, both heels and both soles. The palms were apparently the only localized area not grossly affected by the neoplastic process. The largest of these nodular protuberances was present over the manubrium. It measured 7 cm in width, 4 cm in length and 2 cm in depth. It was immobile and extended into the skin in a small portion of its involved area.

The viscera revealed diffuse neoplastic infiltration. A small, pale yellow nodule was present on the anterior surface of the left ventricle, and microscopic examination revealed that this was composed of the typical young lymphoid tumor cells. The right thoracic cavity was practically entirely replaced by tumor tissue, which involved the wall, the mediastinum, the diaphragm and the small remaining portion of the right lung. The thymus could not be identified as such but rather was part of the large tumor mass extending from the superior mediastinum through the manubrium and forming a large subcutaneous mass over the upper medial area of the chest. The pancreas was also involved with tumor tissue, having an enlarged, irregular, light yellowish white nodule. The adrenal gland was free grossly of any tumor tissue, but on microscopic examination a small discrete tumor nodule was seen occupying a small portion of the cortex and medulla. A small, light yellow subcapsular nodule was present on the surface of the left kidney. There was generalized lymphadenopathy involving primarily cervical, axillary, mediastinal, mesenteric and inguinal glands. The glands had lost their usual configuration and consisted almost entirely of tumor tissue. They were pale yellow, moderately firm and matted. The gross examination of the remaining viscera revealed nothing remarkable. The tumor tissue present in the various organs was of the same morphologic character.

Microscopically, the tumor cells were young lymphoid stem cells and were approximately three to four times the size of the normal adult lymphocyte. The cytoplasm was pale staining, and the cell membrane was not sharply defined. The greater portion of the individual cell, 75 per cent, consisted of the large basophilic lymphoid nucleus, the nuclear membrane of which was sharply defined and stood out above the paler-staining nucleoplasm. Within the nucleus there was an abundance of fine chromatin tissue and a small, clearly demarcated nucleolus. Extending from the cellular membrane and connecting each cell to one or more

neighboring cells were fine argentophilic strands. These intercellular bridges were present throughout the tumor tissue but were more abundant in certain areas. The tumor tissue was not infiltrated by any normal lymphocytes or by inflammatory cells. Careful examination of the neoplastic nuclei revealed occasional mitotic figures. The abundance of reticulum and the intercellular bridge-like strands were seen in all the sections of tumor tissue but were most dramatically present in the specially stained preparations.

Final Diagnosis—Lymphoma, stem cell type, involving the skin, lymph nodes, the right lung, the heart, the diaphragm, the kidneys, the adrenal glands and the pancreas.



A, tumor cells in a lymph node, hematoxylin and eosin, $\times 600$

B, tumor cells in a lymph node, hematoxylin and eosin, $\times 1,500$

C, tumor tissue showing a fine network of reticulum, Wilder's stain, $\times 600$

COMMENT

Gall and Mallory⁷ have proposed the following classification of cancerous lymphomas:

- | | | |
|--------------------------|---|-------------------------|
| 1 Stem cell lymphoma | } | Reticulum cell lymphoma |
| 2 Clasmotocytic lymphoma | | |
| 3 Lymphoblastic lymphoma | | |
| 4 Lymphocytic lymphoma | | |
| 5 Hodgkin's lymphoma | | |
| 6 Hodgkin's sarcoma | | |
| 7 Follicular lymphoma | | |

This nomenclature has been adhered to in this laboratory. To my knowledge, the case reported here is the first publication of a congenital stem cell lymphoma.

The clinical course of the patient is remarkable in a number of respects. At the time of birth an irregular smooth, salmon pink, waxy tumor located over the manubrium and a palpable left axillary lymph node were noted. The mass over the sternum involved the skin as well as the subcutaneous tissue. Clinically the lesion of the skin was suggestive of lymphangioma, but biopsy of the skin revealed changes consistent with leukemia cutis. Gueft and Rosahn¹¹ reported a case of histiocytic leukemia occurring in an infant in whom leukemic infiltration of the skin was noted some ten months before it was manifest in the blood. With this observation in mind my associates and I made serial blood studies of our patient, which failed to reveal any significant abnormalities during life, nor were any observed at autopsy. Sugarbaker and Craver⁶ in their series of 196 cases of so-called lymphosarcoma found involvement of the skin in 55 per cent. Cutaneous infiltration appeared in 16 to 26 per cent of the cases of various types of lymphoma reported by Gall and Mallory⁷ with the exception of those of follicular lymphoma, in which cutaneous infiltration was rare. Neither of these two papers correlates the occasional development of leukemia with the presence of cutaneous lymphomatous involvement.

The initial response of the patient to roentgen therapy was characteristic of cancerous lymphoma. The tumor masses regressed immediately and the infant began to gain weight. The beneficial effects were transitory, and in less than two months the neoplastic process became uncontrollable. The terminal episode was characterized by severe respiratory distress, vital capacity having been markedly reduced by the tumor tissue and the resultant pleural effusion.

The etiology of lymphoma is no more clear than that of the majority of other cancers. While sarcoma and carcinoma have many characteristics in common, they differ in important respects. One of their most remarkable differences concerns the age of onset. Generally, the sarcoma occurs in the middle-aged group and the carcinoma in the older age group. However, as seen in the survey by Wells, the vast majority of congenital cancers have been classified as sarcoma, a group which no doubt includes an unknown number of cancers that might be classified as lymphoma. To date there is no adequate explanation for this prevalence of sarcoma in the fetal and the newborn. The clinically accepted concept that a tumor grows more rapidly in the young has been challenged by the studies of Nettleship,¹² who has transplanted

11 Gueft, B., and Rosahn, P. D. *Am J Clin Path* 13: 516, 1943.

12 Nettleship, A. *Am J Path* 21: 147, 1945.

sarcomatous tissue in animals of different ages and found no correlation between the rate of growth of the tumor and the age of the animal

SUMMARY

A case of congenital lymphoma of the stem cell type has been observed. A review of the pertinent literature indicates that this is the first such case to be published.

NOTE —Because of the editorial policy of the ARCHIVES OF PATHOLOGY, the author's preferred designation, "malignant lymphoma," has been changed throughout to "cancerous lymphoma."

Notes and News

New Journals—*Journal of Gerontology* and *Non-Technical Supplement* (published quarterly, at Springfield, Ill., by Charles C Thomas, publisher, the journal and the supplement are \$6 a year in the United States, Canada and Latin America and \$6 50 elsewhere, the supplement purchased separately is \$3 in the United States, Canada and Latin America and \$3 50 elsewhere) This journal and the supplement are published for the Gerontological Society, Inc. The journal will contain original articles on problems of aging in the field of natural and social sciences and the humanities, reviews and abstracts. The articles in the journal will be abstracted in nontechnical language in the supplement. The supplement will be sent to all regular subscribers of the journal, but yearly subscriptions to the supplement may be purchased separately. The editor is Robert A Moore, 507 South Euclid Avenue, St. Louis 10.

Research in Medical Science The United States Public Health Service announces the early appearance of this new journal—to be published by the National Institute of Health. The journal will be issued monthly, with two volumes to the year. The yearly subscription rates are domestic, \$5, foreign, \$6 75. Subscriptions may be placed with the Superintendent of Documents, Washington 25, D. C. Other correspondence regarding the journal should be addressed to Editor, *Research in Medical Science*, National Institute of Health, Bethesda 14, Md.

Books Received

TOPLEY AND WILSON'S PRINCIPLES OF BACTERIOLOGY AND IMMUNITY Revised by G. S. Wilson, M.D., F.R.C.P., D.P.H., K.H.P., professor of bacteriology as applied to hygiene, University of London, London School of Hygiene and Tropical Medicine, director of the Public Health Laboratory Service, and A. A. Miles, M.A., F.R.C.P., professor of bacteriology, University of London, University College Hospital Medical School. Third edition. In two volumes. Pp. 2,098, with 302 illustrations. Price \$12. Baltimore: Williams & Wilkins Company, 1946.

The first edition (1,300 pages) was published in 1929. In the present revision Professor Miles takes the place of Professor Topley who took no part in the work after 1941 and died in 1944. The division into four parts is maintained: general bacteriology, systemic bacteriology, infection and resistance, the application of bacteriology to medicine and hygiene. The chapter on soil microbiology has been omitted. There are two new chapters, one on antibacterial substances used in the treatment of infections (sulfonamide compounds, penicillin, streptomycin, etc.) and one on the bacteriology of the air. *Shigella* and *Salmonella*, formerly included under *Bacterium*, now have each its own chapter. Chapter 30 on *Salmonella* covers forty-seven pages, four and a half of which are covered by references, one example of the care given to present new developments adequately. The lymphogranulomatous-psittacosis group is put in a chapter by itself. Otherwise the general form of the book is unchanged. At the end of each volume is the same index to both. References to the writings considered in the text are listed under authors' names at the end of each chapter. In most cases these lists

occupy several pages. They are helpful bibliographic guides. The illustrations, some 300, all in black and white, are simple but clear and effective. There are several electron micrographs. The bindings of the volumes seem much too flimsy to withstand the handling they will receive in libraries and laboratories. Part 4 presents in detail the etiology, the morbid anatomy, the epidemiology, the diagnosis, the immunology, the prevention and the treatment of human and animal diseases caused by bacteria and filtrable animal viruses. Obviously, protozoan infections are not considered. Malaria is not even mentioned in the index. This part also has chapters on the normal flora of the human body, on the bacteriology of the air, on the bacteriology of water, shell fish and sewage, and on the bacteriology of milk. The revisers apologize for the increased length of the new edition. "The war has not been conducive to careful leisurely recapitulation, and our plea must be the paradoxical excuse that we have not had time to be more concise." It is hoped that the gap due to the war's interruption of the inflow of periodicals, European and Japanese, can be filled in future editions. All parts of the revision keep well up to the high standards of the previous editions in analytic summarizing of advances on a historical background. Of course avoidable omissions and questionable propositions occur. The American work on poliomyelitis is incompletely reviewed. The congenital deformities occurring in the offspring of mothers who had rubella in the early months of pregnancy have missed mention. The difference between the scarlatinal and the erysipelatoïd streptococcus toxins is still not brought out clearly enough. The inclusion of pneumococcus as *Streptococcus pneumoniae* as a genus under the type species of *Streptococcus pyogenes* will excite new interest in bacteriologic classifications. The book continues to be an at present unequalled source of explanatory information about pathogenic bacteriology. It is a cyclopedia of facts as well as a discourse on principles.

SPEZIELLE CHIRURGISCHE THERAPIE FÜR STUDIRENDE UND ÄRZTE. Bearbeitet von Dr. Max Saegesser, Privat-dozent für Chirurgie, Bern. Pp 884, illustrated. Price, Swiss francs 80. Bern, Switzerland. Medizinischer Verlag Hans Huber, 1946.

ELECTROCARDIOGRAPHY IN PRACTICE. By Captain Ashton Graybiel, M.D., United States Naval Reserve Co-Ordinator of Research, United States Naval School of Aviation Medicine, Pensacola, Florida, and Paul D. White, M.D., lecturer in medicine, Harvard Medical School, physician, Massachusetts General Hospital, with the assistance of Louise Wheeler, A.M., executive secretary, Cardiac Laboratory, Massachusetts General Hospital, and Conger Williams, M.D., assistant in medicine, Harvard Medical School and Massachusetts General Hospital. Second edition. Pp 458, with 323 illustrations. Price \$7. Philadelphia and London. W. B. Saunders Company, 1946.

STUDIES IN HYPERTONY AND THE PREVENTION OF DISEASE. By I. Harris, M.D., honorary director, Institute for Prevention of Disease, and honorary physician, Liverpool Heart Hospital, in cooperation with J. T. Ireland, G. V. James, Edward Cronin Lowe and C. E. Vernon. Pp 114. Price \$3. Baltimore. Williams & Wilkins Company, 1946.

AN EXPERIMENTAL STUDY OF RATIONING. By R. A. McCance and E. M. Widdowson. Medical Research Council Special Report Series no 254. Pp 61. Price, 30 cents. London. His Majesty's Stationery Office, 1946.

THE MODERN ATTACK ON TUBERCULOSIS. By Henry D. Chadwick, M.D., and Alton S. Pope, M.D. Revised edition. Pp 134. Price \$1. New York. The Commonwealth Fund, 1946.

RADIATE FORMATION ON PATHOGENIC FUNGI IN HUMAN TISSUE

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THE APPEARANCE and the nature of pathogenic fungi seen in tissue have been for the most part well described. Of particular interest is the finding, on pathogenic fungi in tissue, of the peculiar phenomenon known as radiate formation. This appearance of radiation has come to be accepted as a commonplace with various organisms, notably species of *Actinomyces* or *Nocardia* (ray formation) and *Coccidioides immitis* (prickles). Such radiate formation is quite commonly encountered in experimental aspergillosis (*Aspergillus fumigatus*) in laboratory animals but is still a rarity in man. In certain cases of sporotrichosis a radiating substance has been seen in tissue of man—but only in a very few instances. Why these formations occur has never been satisfactorily explained. The views and theories advanced have been many and diversified. Attention was drawn to this structure recently when radiate formation was observed on cells of *Sporotrichum schenckii* in tissue¹ and on cells and filaments in 2 human cases of aspergillosis. As a result, an attempt is being made to determine just why such structures are formed and under what conditions. This paper will be devoted to the presentation of such organisms with radiate formation as have been found in human tissue, the histopathologic picture within which such structures are seen and the prevailing theories which have been advanced in the effort to explain the phenomenon.

ACTINOMYCOSIS

Definition—Actinomycosis is a local or systemic disease, granulomatous in nature, and may be acute, subacute or chronic. It is characterized chiefly by sinuses and fistulas from which may be isolated variously colored granules which are masses of mycelium of species of

From the Laboratory for Mycology, Department of Dermatology of the Barnard Free Skin and Cancer Hospital, service of Dr M F Engman Sr

1 Moore, M, and Ackerman, L V Arch Dermat & Syph 53 253, 1946

the genus *Actinomyces* (*Nocardia*, *Discomyces*, *Streptothrix*, *Actinobacillus*, *Cohnistreptothrix*, *Brevistreptothrix* and *Pioactinomyces*)

History—The disease as it occurs in man is usually considered to have been first described by Israel² in 1878, when he observed the radiating tungus in the pus of a patient suffering with empyema. This followed what was considered the original observation of the infectious process known as "lumpy jaw" in cattle and described by Bollinger³ in 1877. The organism in cattle was described and named by Harz (in Bollinger's report) *Actinomyces bovis*. In 1885 Murphy⁴ reported the disease in America for the first time, three years after Ponfick⁵ had shown the similarity of the infections in man and animal.

Negroni and Bonfiglioli⁶ have credited Lebert, of Paris, France, with having been the first to observe this disease in man, in 1848. Israel in his publication of 1878, however, reported that he showed his preparation to Professor von Langenbeck, who recalled a case seen by him in 1845 at Kiel, Germany. Israel included in his paper a résumé of the case described by von Langenbeck. His figure 9c shows the characteristic radiate formation of *Actinomyces* which von Langenbeck described as *cylindrische Körperchen von eigenthümlicher Lichtbrechung* (cylindric bodies of a peculiar refraction of light). It is apparent, therefore, that credit should be given to von Langenbeck for the observation of the first human case of actinomycosis showing radiate formation.

Pathology—Grossly, actinomycosis of the skin manifests itself in the form of nodules which ulcerate to produce suppuration and sinus formation and which may result in scar tissue. The sinuses become intercommunicating to produce eventually a large tumefaction with granulation tissue and multiple sinuses exuding seropurulent or sero-sanguineous material onto the skin surface. In the internal organs, the nodules rupture, the pus is spread through the tissue, attacking bones as well as soft tissue, and abscesses develop which eventually form the sinuses described.

Microscopically, the lesions of actinomycosis are granulomatous in nature. At the early stage a small nodule develops, with the organism in the center of the node. The area is infiltrated by lymphocytes, polymorphonuclear leukocytes, eosinophils and large, irregular macrophages. This area in turn is surrounded by plasma cells and proliferating con-

2 Israel, J. *Virchows Arch f path Anat* **74** 15, 1878

3 Bollinger, O. *Centralbl f d med Wissensch.* **15** 481, 1877

4 Murphy, J. B. *New York M J* **41** 17, 1885

5 Ponfick, E. *Die Actinomykose des Menschen, eine neue Infektionskrankheit auf vergleichend-pathologischer und experimenteller Grundlage geschildert*, Berlin, A. Hirschwald, 1882, p. 138

6 Negroni, P., and Bonfiglioli, H. *J Trop Med & Hyg* **40** 206 and 240, 1937, *Rev Soc argent de cien nat* **15** 159, 1939

nective tissue cells The connective tissue surrounding the whole area becomes noticeably edematous and is infiltrated by polymorphonuclear leukocytes and lymphocytes As the organism grows, the bacillary forms develop filaments, which become intertwined The cells around the fungous elements show degenerative changes and are finally replaced by invading leukocytes The result is a central area of fungous elements which are intertwined and compact and which may show either radiate or "club" formation or simply a mass of filaments This is the granule seen in smears of pus The granule is surrounded by various leukocytes, many showing degenerative changes and cellular debris The edematous connective tissue surrounding this area appears as granulation tissue Macrophages, many showing phagocytosed fat, can be seen in the neighborhood, and some of these large cells may invade the pyogenic mass The nodules may remain discrete, or they may coalesce to form large masses As this large necrotic area increases in size and forms a frank abscess, the pus seeks more space and consequently burrows through the adjacent tissue The path created by the pus constitutes a sinus The sinuses do not heal easily because of the constant flow of pus, with the result that numerous such sinuses may be formed, many becoming intercommunicating Large pockets of pus may develop along the path of the sinus The sinus eventually becomes filled in with granulation tissue, which may show scattered leukocytes and numerous newly formed blood vessels and young connective tissue cells

Fungus in Tissue—The organisms of actinomycosis may be divided into two main groups, the aerobic and the anaerobic or microaerophilic The aerobic forms, which some workers regard as a distinct biologic group and therefore classify as *Nocardia*, exemplified by *Nocardia asteroides*, is made up of both pathogenic and saprophytic organisms, some having acid fastness as a property The second group, the anaerobic or microaerophilic forms of *Actinomyces*, is considered by some as being made up of a single species, *Actinomyces bovis*, or, as preferred by Dodge⁷ and also by Negroni, *Actinomyces israeli* This is the pathogenic actinomycete commonly encountered in human tissue, which is gram positive and non-acid-fast and is chiefly responsible for the so-called sulfur granules

In spite of the controversy over the classification of *Actinomyces*, in tissue the fungus in its late stage may be seen as a granule, which may be white, whitish yellow, black green or red and may or may not show the clubbed or radiate formation (fig 1, 1 and 2) When *Actinomyces* first invades human tissue, it does so either in the form of a bacillus-like cell, which is approximately 0.2 to 0.6 micron in diameter and somewhat irregular in form, or as nonseptate, occasionally branching filaments, which vary in length but are approximately

⁷ Dodge C W Medical Mycology Fungous Diseases of Man and Other Mammals St Louis C V Mosby Company 1935 p 714

15 microns The fungus in the early form is carried through either the blood or the lymph stream and comes to rest at some locus in which, if the conditions are favorable to it, the organism grows and multiplies

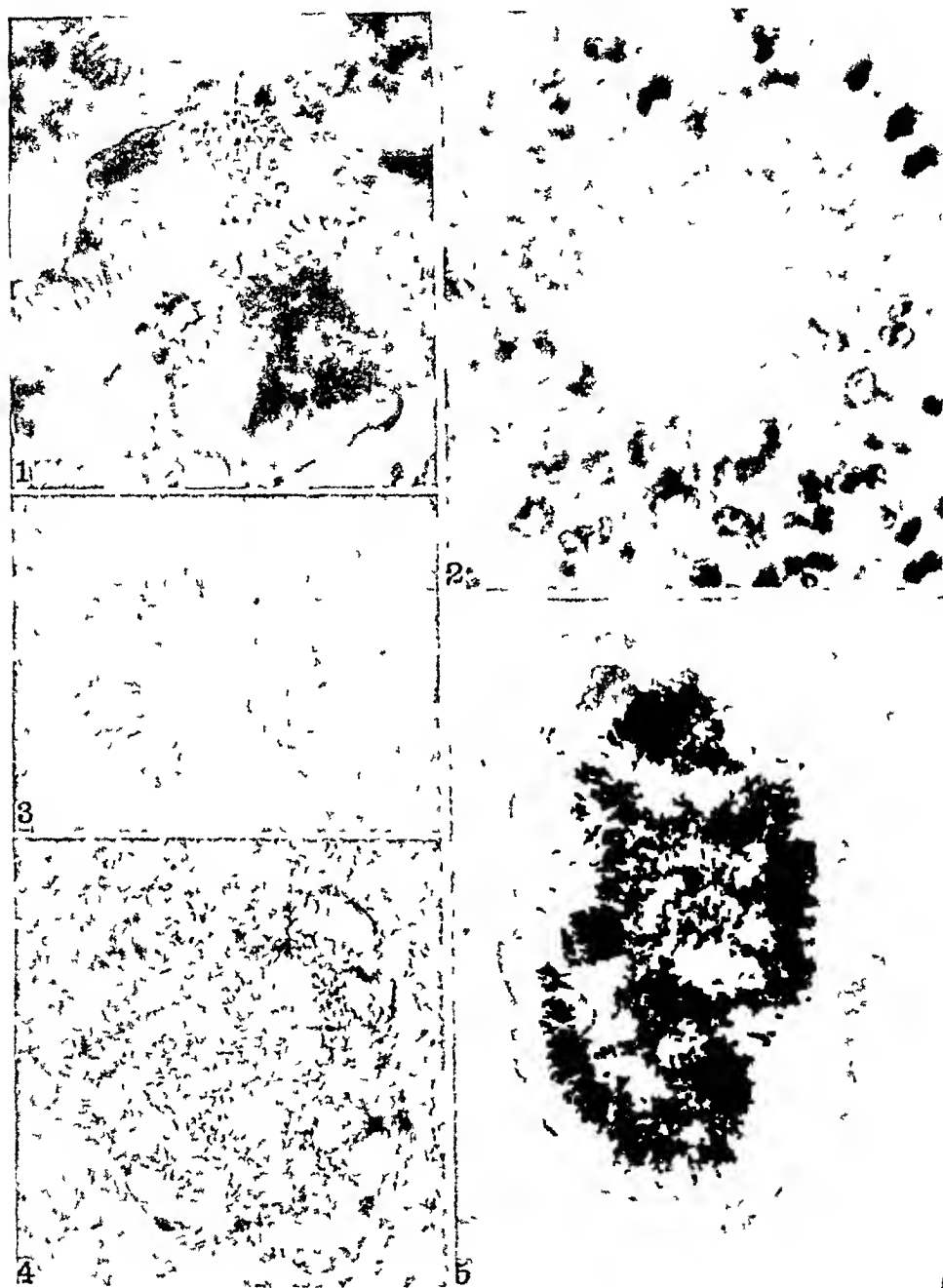


Fig 1—1 Radiate formation of actinomycotic granules, hematoxylin and eosin, $\times 558$ 2 Actinomycotic granule minus clubs in the brain, hematoxylin and eosin, $\times 988$ 3 Soft radiating granule of *Actinomyces* in pus, $\times 1052$ 4 Hard granule of *Actinomyces* showing lobulate arrangement, seen in pus, $\times 574$ 5 Actinomycotic granule showing organisms and radiate formation, seen in skin, Gram-Weigert stain, $\times 446$

The bacillary form or the filaments elongate, branch and become intertwined and compact to make up the young granule. The growth process continues and, as a result, there develops one of two types of granules, the soft or the hard. The soft granule may show in its center a few degenerate polymorphonuclear leukocytes or leukocytic granules. The granule proper consists of filaments which may be intertwined but have peripheral extensions (fig 1, 3). The young hard granule when freshly isolated is small and has a central region consisting of leukocytes or leukocytic granules and intertwined filaments. Extending from the periphery in the form of rays, and usually covering the whole granule, is a hyaloid or gelatinous-like substance. At the periphery of the granule these extensions appear to engulf the filaments of the fungus, with the terminal portions appearing filiform, flattened or, usually, swollen or club shaped. When the granule is older and when placed on a glass slide, it is usually resistant to crushing, which often causes the cover slip to be broken. These older granules appear to have the consistency of small calcified nodules. When examined microscopically, they are seen to consist of lobulated masses (fig 1, 4). The central area is amorphous in nature, while the lobulated periphery is hard and glasslike in appearance, refractile and made up of radiating rays or clubs. Some of the radiations may appear either as flattened, broad or as needle-like projections, with a yellowish tinge, and seem very refractile. When stained with hematoxylin and eosin, the center of the granule shows leukocytes or leukocytic and pigmentary granules, which stain heavily. Surrounding this is the amorphous-like material consisting of filaments and some degenerate cells, which may also stain deeply with the hematoxylin. The periphery of the granule, consisting of the radiations or clubs, takes the eosin stain and is said to be acidophilic. These peripheral extensions were thought to be merely the growing, swollen tips of the fungus since in some cases they appeared to be closely adherent to the filament. When stained by the Gram-Weigert method, however, the filaments of the fungus are gram positive, while the clubs take the eosin stain, showing that the two are distinct entities (fig 1, 5).

ASPERGILLOSIS

Definition—Aspergillosis as a disease of man is caused by species of *Aspergillus* with the production of chronic inflammatory and granulomatous lesions of the pulmonary system, the external auditory canal, the mucous membrane of the conjunctiva, the cornea, the nasal sinuses, the kidney and occasionally of the skin, the nails, the bones and the meninges.

History—The first description of a human case of mycosis which may be doubtfully considered as one of aspergillosis is that attributed to

Bennett, who in 1842 found fungi in the sputum of a patient Rénon⁸ contributed a comprehensive review of the early literature with a study of aspergillosis in 1897, and he credited Sluyter with being, in 1847, the first to describe a case of pneumonormycosis actually caused by *Aspergillus*. The patient was a woman who had died of a pulmonary infection. In a cavity of the lung, Baum, Litzmann and Eichstedt found black, adherent masses made up of mycelial filaments and round bodies. Some of the filaments emerging from the mass had swollen extremities on which these observers noted a large number of ovoid cells. They considered the organism a *Mucor*, but Virchow, who studied the material in detail, believed that it was probably an *Aspergillus*.

The first scientific report of aspergillosis was made by Virchow⁹ in 1856, when he reported 4 cases of bronchomycosis and pneumonormycosis due to *Aspergillus*. The 4 patients had died, respectively, of dysentery, pulmonary inflammation with emphysema, carcinoma of the stomach and pneumonia. Reports of a number of other cases followed, and these have been reviewed by Rénon (1897), by Lang and Grubauer¹⁰ (1923) and by others.

The first observation of the radiate or actinomycetoid form of *Aspergillus* in tissue was made by Lichtheim¹¹ in 1882 when he injected *Aspergillus fumigatus*, obtained from the lung of a woman at necropsy, into a rabbit. In 1884 Laulané¹² observed the radiating forms in the lung of a rabbit and described them as having the appearance of a rosette, with the very short extensions yellow, refractile, swollen at the free end and needle shaped at the adherent end. These forms have since been observed in numerous animals by many investigators, both in spontaneous and in experimentally induced aspergillosis.

The radiating form of *Aspergillus* was described as it occurs in man, perhaps for the first time, by Wheaton¹³ in 1890. He observed a case of pneumonormycosis involving not only the lungs and the bronchi but also the lymph nodes. The patient was a 2½ year old girl who had a slight cough and showed a loss of weight for two months. Wheaton found small cavities filled with pus, and in the surrounding tissue there were bright orange-colored bodies the size of mustard seeds. The rosette-like bodies seen in the lung, as illustrated in the accompanying

8 Renon, L. *Etude sur l'aspergillose chez les animaux et chez l'homme*, Paris, Masson & Cie, 1897, p 300

9 Virchow, R. *Virchows Arch f path Anat* **9** 557, 1856

10 Lang, F J, and Grubauer, F. *Virchows Arch f path Anat* **245** 480, 1923

11 Lichtheim, L. *Berl klin Wchnschr* **19** 129 and 147, 1882

12 Laulané, F. *Arch de physiol norm et path* **4** 487, 1884

13 Wheaton, S W. *Tr Path Soc London* **41** 34, 1890

woodcut, were the characteristic radiating structures. A *fumigatus* was obtained in culture. In 1893 there appeared two reports on the actinomycetoid form of *Aspergillus* in human tissue. One, by Boyce,¹⁴ described macrophages of varying size which engulfed masses of hyphae. In describing the structures, he pointed out that "stains have very little effect upon them, the most powerful nuclear dyes rendering the nuclei only just visible, the cell substance has a very characteristic yellow tinge, and typical amoeboid outline, the pseudopodia may be long and blunt, and often have a ground-glass appearance, which contrasts with the clearer and more granular condition of the rest of the cell." His illustration of a nodule in the lung showed little, if any, of the hyaloid, ground-glass-appearing substance but showed mostly radiating filaments of the fungus, such as one sees in culture. However, other illustrations depict brownish encrusting substances on the filaments, some in the form of swellings and others looking as if they were macrophages, which were similar to some seen in a case which will be described. The other paper published in 1893, by Kohn,¹⁵ described granules in lung tissue in a case of aspergillar pneumomycosis which were similar to those of actinomycosis and from all sides of which long slender filaments extended outward.

The occurrence of *Aspergillus* as an agent of mycetoma has been recognized in the literature. The occurrence of the organism in this disease, however, is rare. In 1930 da Fonseca¹⁶ reported a case of Madura foot caused by *Aspergillus amstelodami*. Biopsy of the tissue revealed numerous hard, rounded granules, many having their surfaces covered with extensions. The granules were sulfur yellow to green. His microscopic observations of the granules and the accompanying illustrations are in keeping with the characteristic radiating aspergillotic granules.

Two unreported cases of aspergillosis of the lungs with radiate formation have been seen and diagnosed as such by me. The first case was that of a patient at the United States Marine Hospital at Staten Island, N. Y. Slides of tissue made from a localized lesion of the lung were received in 1942 from Dr. J. A. Pasternack, at that time director of pathology. The sections consisted almost entirely of fungous elements, with little tissue. In one area of the section there were masses of filaments which seemed to be undergoing hyalinization, leading to the ground-glass-like appearance. These masses took the eosin stain except for some areas which did not become colored but

14 Boyce, R. J. *Path. & Bact.* **1** 163, 1893.

15 Kohn, H. *Deutsche med. Wchnschr.* **19** 1332, 1893.

16 da Fonseca, O., Jr. *Rev. med.-cir. do Brasil* **38** 415, 1930.

appeared yellowish and refractile. In this zone were seen the typical radiating structures of *Aspergillus*.

The second case was that of a 29 year old man seen on the chest service of Dr. Evans Graham at Barnes Hospital, St. Louis. The patient had noted recurrent hemoptysis for the past fifteen years, which became worse on exertion. A roentgenographic study disclosed an infiltration of the middle lobe of the right lung, and a bronchogram showed an obstruction in the right middle lobe bronchus. Bronchoscopy revealed partial stenosis of the right middle lobe bronchus and a small amount of blood and pus coming from the orifice of this lobe. The right lung was removed. An area of definitely diseased lung was found in the region usually occupied by the middle lobe, but actually it was part of the upper lobe of the right lung. It consisted of contracted scar tissue with some surrounding atelectasis and inflammatory infiltration. Five millimeters beneath the area of adhesions and fibrin there was a thick-walled abscess cavity, which was diagnosed as a chronic abscess of the lung. The cavity was 12 mm. in diameter, with a hard, white, resilient wall. The inner wall of the cavity was covered with soft, friable, brown material. The parenchyma distal to the cavity in the upper lobe showed fibrosis and hemorrhage as well as dilatation of the bronchi. On microscopic examination, masses of fungi and inflammatory cells were seen in the cavity and in the dilated bronchi. In the surrounding parenchyma there was considerable fibrosis, as well as a giant cell reaction, many of the giant cells phagocytosing fungous filaments. The diagnosis of chronic abscess of the lung and aspergillosis was made.

Pathology—Aspergillosis is usually characterized by the presence of edema and erythema. The inflammation and the inflammatory response may be so pronounced as to bring about very noticeable changes in the affected and the surrounding tissue. Pulmonary involvement is perhaps the most common of the infectious processes. In the lungs the lesions are chiefly of the inflammatory or tubercle-like types. Small white to yellowish white nodules may be seen grossly in the involved areas. The involvement may be parenchymatous or interstitial. In parenchymatous aspergillosis the mucosa of the bronchi when involved may show congestion with or without ulceration and membranous patches. Lesions of the alveolar sacs may remain localized with the formation of tubercle-like structures and abscess-like accumulations of cells or they may become widespread and generalized. The abscesses are usually seen in the smaller bronchi, from which they may push through to involve the lung tissue. The pleural surface may become infected, and inflammation, congestion, thickening and fibrosis follow, with resultant pleuritic pain.

Interstitial aspergillosis usually does not remain localized, it tends to involve the alveoli, reproducing the pathologic processes noted in the foregoing paragraph. Thrombi may be formed in the infected arterial lumens, and atheromatous patches may be seen throughout the arterial tree, in bronchopulmonary involvement.

Microscopically, the cellular infiltrate is similar in many respects to that seen in other mycotic granulomas. The infiltrate may be diffuse, or it may be circumscribed, as in the case of localized, nodular lesions. Interspersed among or surrounding the mass of fungous filaments may be seen leukocytes, leukocytic granules, lymphocytes, plasma cells and giant cells of the Langhans type. Central necrosis may or may not be present, while the whole response may be somewhat encapsulated with fibrous material. The giant cells may be few or many and often are arranged in tubercle-like fashion. Many of the giant cells engulf fungous elements. In some of the giant cells can be seen yellowish, nonstaining, refractile elements, which appear to be not the fungous filaments described but the hyaloid, encrusting or radiating substance seen in the radiating granules.

Fungus in Tissue—*Aspergillus* assumes various forms in tissue and thereby differs greatly from some of the common pathogenic fungi. In culture the fungus is characterized by the production of filaments, spores of various types and spore heads or conidiophores with the development of conidia. Except in aural infections and perhaps experimentally produced disease, the conidiophores are not often seen in human tissue. The ear, with its moistness, warmth and excreta, serves as an excellent culture medium for the growth of fungi and may well be compared with a test tube or a culture plate. The conidiophores grow abundantly and luxuriously on the surface. The penetrating organism, however, consists chiefly of filaments and large round or ovoid spores, perhaps comparable to the chlamydospores seen in cultures. In organs in which there is not the amount of free air present that one finds in the ear, the fungus is seen to consist chiefly of branching, interlacing, septate hyphae or filaments. In the lung, where the organism may be found in large masses either free in the tissue or in bronchi or bronchioles, there can be seen layers of large spherical to ovoid cells adjacent to the peripheral filaments of the growing mass. These are the so-called chlamydospores.

The radiate structure on *Aspergillus* in tissue also assumes various forms. It may be seen on a single, thick-walled (double-contoured), spherical cell or spore, on a short or a multicelled filament or as extensions on a granule made up of a mass of filaments and spores. The simplest form shows a central thick-walled spore with peripheral extensions (fig 2, 1). These radiations may be somewhat uniform in

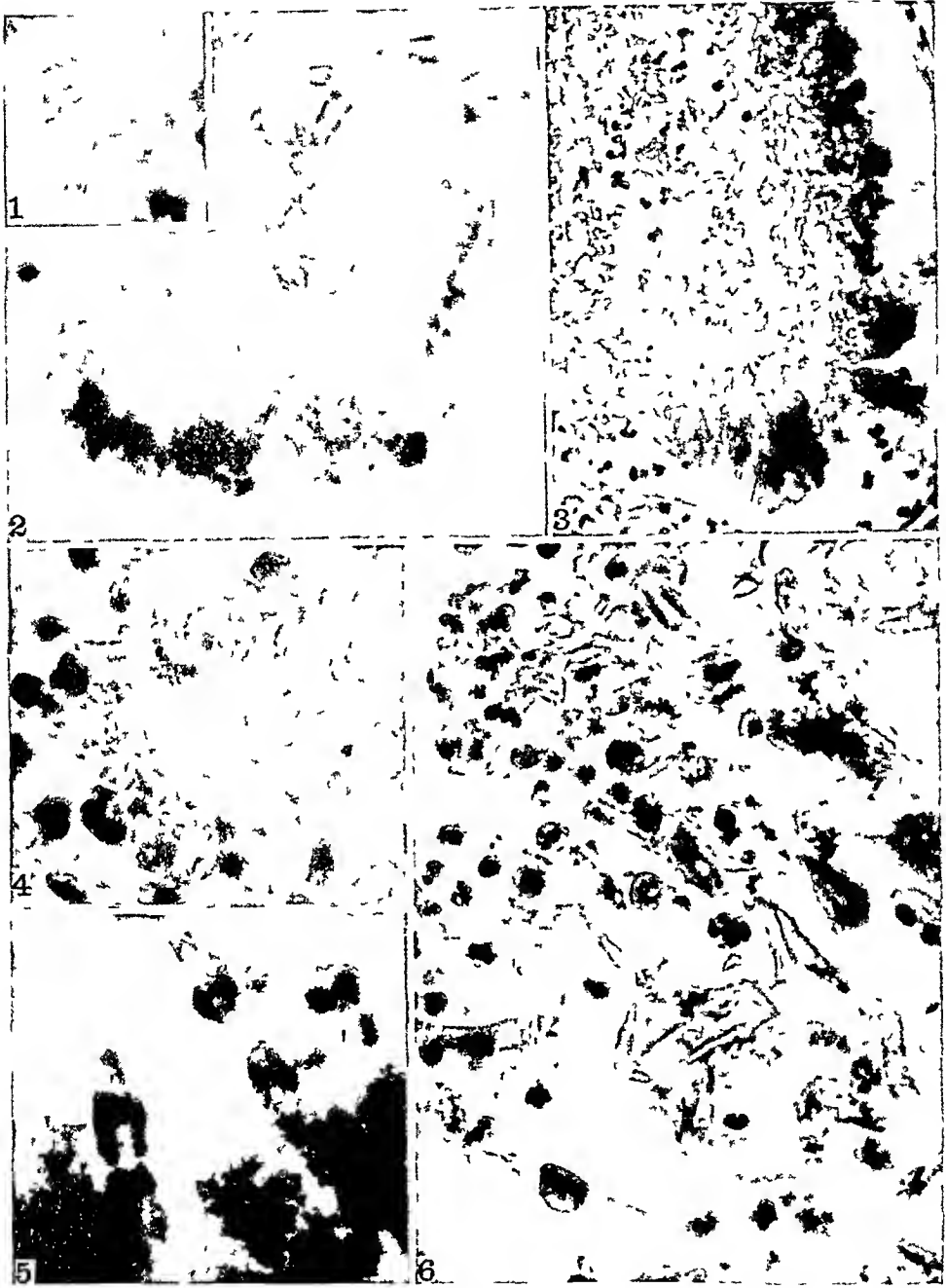


Fig 2—1 Radiate formation on a single cell of *Aspergillus*, hematoxylin and eosin, $\times 1,110$ 2 Radiate formation on a branching hypha of *Aspergillus*, hematoxylin and eosin, $\times 1,110$ 3 Periphery of aspergillotic granule showing acidophilic material, seen in lung, hematoxylin and eosin, $\times 350$ 4 Aspergillotic granule in lung, showing ground-glass-like appearance with radiating crystalline-like substance, hematoxylin and eosin, $\times 853$ 5 Incrustations on hyphae of *Aspergillus*, hematoxylin and eosin, $\times 1,222$ 6 Incrustations on cells and filaments of *Aspergillus*, hematoxylin and eosin, $\times 510$

size and shape, or they may be irregular, at any rate they suggest the asteroid type described by others. The rays are hyaloid to ground-glass-like in appearance, they may stain with eosin, or they may remain unstained, in which case they are yellowish to light yellowish green (fig 2, 2). The tips of the rays are not swollen or rounded, as in the club formation seen in actinomycotic rays, but appear more as elongated crystals with "broken-off," or abrupt tips. In some cases the radiations appear more as a uniform crystalline mass with short angular projections or as segmented forms. In some cases the radiations appear completely around the central fungous cell, giving the whole the appearance of a spiny ball (fig 2, 4). The radiations on the filaments are somewhat similar in that they appear along the length of the filament in much the same fashion as around the spherical cell. They usually are of unequal length, appear at times like crystalline needles, although somewhat greater in diameter, but in general conform to the tips already described.

The granules when seen in tissue vary somewhat in size but usually are 1.5 mm in diameter, they are hard, yellow to yellowish green and not lobulated, as are the actinomycotic granules. Radiations may or may not be present, but usually are rare. The nonradiating granule is a compact mass of intertwining septate filaments. Toward the periphery of the granule can be seen large spherical to ovoid cells, the chlamydospores. These cells may be seen at times in the form of a layer. The periphery of the granule is made up of loosely interwoven hyphae, scattered among which may be seen many leukocytes, chiefly polymorphonuclears, and other inflammatory cells. The granules with peripheral extensions or radiations when seen in cut sections stained with hematoxylin and eosin are composed of three zones. The central area is made up of compactly interwoven filaments which stain lightly. Surrounding this is a zone which stains more intensely with hematoxylin and also shows a substance which appears to be in the form of an incrustation on the hyphae and which stains deeply as if with a mixture of hematoxylin and eosin. This zone is more or less amorphous in appearance and apparently is fairly brittle, since one sees cracking, which is absent in the central zone. When this area is examined under higher power, filaments and chlamydospores can be discerned within it. Surrounding the midzone is an area of loosely interwoven hyphae with many of the large spherical to ovoid cells capped by the eosinophilic or acidophilic staining, irregular radiations or ray formations (fig 2, 3). These may or may not surround the entire granule. Usually, however, in cut sections they may be seen involving only part of the periphery, with the uninvolved areas consisting of masses of filaments engulfed by inflammatory types of cells as noted in the

nonradiation granules. One may also see at various points within the granules areas made up entirely of the eosinophilic substance, whereas furrows or spaces may be seen which are devoid of fungi, being filled instead with leukocytes.

In addition to the radiating types described, there is another type of formation which has been observed in 1 of the cases noted and which Boyce described and illustrated in his paper as a macrophage (see his fig 6). These "macrophages" stain with eosin, or they may remain unstained, and have a yellowish color and appear to invest or engulf a whole or a part of a hypha or groups of hyphae, or they may appear as one to several nodules on a hypha. Their appearance is more that of an incrustation or concretion than that of a macrophage (fig 2, 5 and 6). On closer examination, however, it is apparent that the engulfing substance is similar to, if not identical with, that of the radiating forms seen on other filaments and granules. In 1925 Dillard and Weidman¹⁷ described 2 cases of multiple hemorrhagic sarcoma of Kaposi. In the second case the patient, an 82 year old laborer, was admitted to the hospital with bronchopneumonia. He died twenty-four hours after admission, and necropsy was performed. When the gastrohepatic lymph node was being examined, there were seen filaments or mycelia, both free and in giant cells (see their figs 13 and 14), which were described as "homogeneous, highly refractile and hyaloid." It is apparent from the description of both the fungi and the histopathologic appearance of the lesion that the fungus was *Aspergillus*. In their figure 14 are illustrated fungous cells with thick coatings and "chlamydospores" which certainly correspond to the radiating forms described in this paper. It is probable that the forms described by Weidman and Douglas¹⁸ in a paper published in 1921 may likewise have been such radiating forms of *Aspergillus* (see their fig 10). In 1932 Weidman¹⁹ compared the "chlamydospores" seen in the case published in 1925 with the Hülle cell of *Aspergillus*, as described and illustrated by Thom and Church, who refer to the work of Eidam. As Weidman correctly put it, the Hülle cell is a thick-walled cell of an aborted reproductive structure which actually is comparable with the true chlamydospores formed under adverse conditions of growth. This being the case, it is doubtful whether the structures seen by Weidman could be Hülle cells, since reproductive structures in parasitized tissue are not the rule and, further, the engulfing substance was found on filaments as well, which certainly could not be interpreted as fruiting bodies.

17 Dillard, G. J., and Weidman, F. D. *Arch. Dermat. & Syph.* **11**: 202, 1925.

18 Weidman, F. D., and Douglas, H. R. *Arch. Dermat. & Syph.* **3**: 743, 1921.

19 Weidman, F. D. *Arch. Path.* **13**: 725, 1932.

COCCIDIOIDOMYCOSIS

Definition—Coccidioidomycosis is a mycotic infection caused by *C. immitis*, which may manifest itself in two chief forms (a) a respiratory disease, which may be acute, self limited in duration and benign, and (b) a chronic, progressive granulomatous form—coccidioidal granuloma—with remissions, relapses and widespread dissemination (systemic), involving skin and visceral and osseous structures

History—The disease was first described in 1892 by Posadas²⁰ from Buenos Aires, Argentina, as a new case of mycosis fungoides. In the same year, Wernicke²¹ reported this case in another journal. Two years later, the second instance of the infection was reported in the United States by Rixford²². In 1896 Rixford and Gilchrist²³ published in detail the second and also the third case of the infection. The organism, at first considered to be protozoon, was named *Coccidioides* by Stiles, a medical zoologist. However, the microbe was found to be a fungus and now bears the name *C. immitis*. Up to the present time a large number of cases of the disease have been discovered, and these chiefly in such endemic regions as the San Joaquin Valley, Calif., and in parts of Texas, New Mexico and Arizona. In Argentina the incidence of the disease is still extremely low.

The first observation of radiate formation on cells of *C. immitis* in human tissue was made perhaps by Rixford and Gilchrist in 1896, when they figured a cell (see their fig. 17) with the description that the "parasite presents a number of fine prickles." These prickles extended out from the capsule. In 1926 Ahlfeldt²⁴ called attention to these radiations in experimentally produced disease in guinea pigs, stating that she found the prickles in several sections (see her fig. 1) and that they were found only in adult organisms that were ready to liberate young forms or the spores. In a later publication²⁵ there are drawings also of radiating forms of *C. immitis*. In 1932 de Almeida observed radiate forms of *C. immitis* in guinea pigs experimentally infected with this organism. These observations were made in 3 animals with three different strains of the fungus. Radiate formation was seen by de Almeida in 2 human cases of the disease. The radiation effect on cells of *C. immitis* in human tissue has since been noted by others.

20 Posadas, A. *Ann. d. Circ. med. argent.* **15** 585, 1892.

21 Wernicke, R. *Centralbl. f. Bakt. (Abt. 1)* **12** 859, 1892.

22 Rixford, E. *Occidental M. Times* **8** 704, 1894.

23 Rixford, E., and Gilchrist, T. C. *Johns Hopkins Hosp. Rep.* **1** 209, 1896.

24 Ahlfeldt, F. E. *Arch. Path.* **2** 206, 1926.

25 Ahlfeldt, F. E. *J. Infect. Dis.* **44** 277, 1929.

Pathology—The reaction of the tissue to *C. immitis* in the progressive or granulomatous form of the disease is similar in many respects to that noted in other mycotic granulomas. Microscopically, the skin appears acanthotic, with pronounced parenchymatous or interstitial edema and vesicles in the epidermis. Abscesses may be seen throughout the region involved in the inflammatory process, but these do not, as a rule, reach the large proportions of those noted in blastomycosis. The nodular type of lesion usually suppurates and becomes necrotic, but definite large abscesses are rarely formed. Where the fungus is found, however, abscesses may be very apparent, as well as an inflammatory response. In the dermis there is a granulomatous response with an infiltration and proliferation of lymphocytes and of plasma, epithelioid and giant cells the last of the Langhans type. Newly formed blood vessels are apparent, and there is a tuberculoid response with the formation of tubercles which may show caseation and liquefaction necrosis followed by fibrosis and calcification. The edema in the epidermis may extend into the corium and the subcutis, while in other cutaneous lesions the hyperplastic epidermis may be accompanied by whorl and pearl formation not unlike that seen in a carcinomatous process.

In its systemic spread the organism may cause the formation of abscesses, areas of necrosis and granulation tissue. In the lungs the lesions may simulate grossly those of miliary bronchopneumonia or bronchiolitis, acute or chronic miliary tuberculosis, miliary carcinoma-tosis or secondary stage silicosis. Induration, cavitation and caseation may result. The disease is less marked in the region of the mediastinum than in the periphery of the lung field. Lesions are usually not present in the esophagus and the small intestine. The microscopic observations in the systemic spread are quite similar to those in the cutaneous involvement, with noticeable edema, infiltration and proliferation of the various cells already named, necrosis, tubercle formation, caseation, liquefaction and, finally, fibrosis and calcification.

Fungus in Tissue—In tissue, pus, pleural fluid, sputum or exudates *C. immitis* is seen as a thick-walled spherical structure, measuring from approximately 2 to 80 microns in diameter, and may be either a simple, nonbudding cell or a large sac filled with endospores. In its host the fungus reproduces by endospore formation. The spores are set free by rupture of the wall of the mother cell. The spores enlarge, endospores are formed, and the process repeats itself. In the normal adult form the endospores are many. Occasionally, there may be large cells containing only a few spores, and they are generally considered to be immature.

Radiate formation on cells of *C. immitis* in tissue varies from small prickles, as described by Rixford and Gilchrist and by Ahlfeldt,

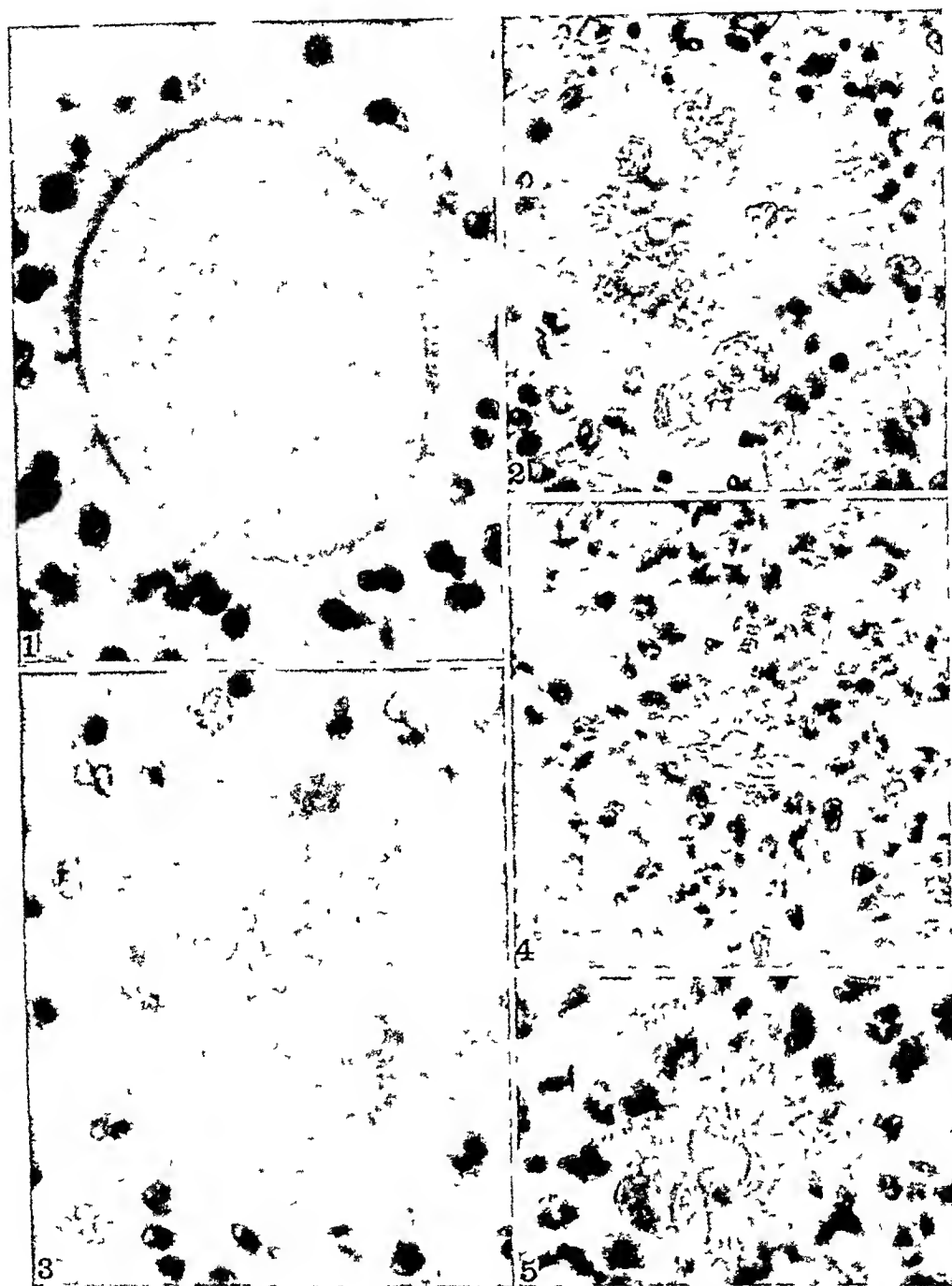


Fig 3—1 Endosporulating cell of *Coccidioides immitis* showing prickle formation, hematoxylin and eosin, $\times 1,110$ 2 Group of spores and mother cells of *C immitis* with radiating acidophilic material, hematoxylin and eosin, $\times 558$ 3 Endosporulating cell of *C immitis* showing club formation, hematoxylin and eosin, $\times 972$ 4 Radiation on spherical cell of *Sporotrichum schenckii*, methylene blue and eosin, $\times 669$ Note the leukocyte infiltrate 5 Radiations on a thick-walled spherical cell of *S schenckii* showing abrupt, "broken-off" tips of rays, methylene blue and eosin, $\times 1,110$

to large radiating forms. The prickles may be fine or broadened, measuring approximately 2 to 5 microns (fig 3, 1). Ray forms may be seen as elongated, ovoid structures, varying in length and simulating actinomycotic radiations, or they may be rather long tubular projections, such as those described by de Almeida (fig 3, 3). Occasionally, the eosinophilic radiations can be found extending from a group of spores of an apparently freshly ruptured mother cell, or they may appear to be engulfing groups of spores as well as immature mother cells (fig 3, 2). In all observations of radiating cells of *C. immitis* as in the case of both *Actinomyces* and *Aspergillus*, the microscopic picture of the tissue response remains constant. These structures are seen in areas heavily invaded by leukocytes and by cells of an inflammatory type and are in close contact with these cells.

SPOROTRICHOSIS

Definition—Sporotrichosis is a granulomatous process which is usually confined to the cutaneous or the subcutaneous tissue. The organisms, members of the genus *Sporotrichum*, may spread through the lymph channels to produce various lesions of the skin, or they may involve the internal viscera and bony structures.

History—The first pathogenic species of *Sporotrichum* was probably that described by Montagne.²⁶ The organism was isolated from a case of bronchomycosis by Gubler and was named *Sporotrichum bronchiale*. The important knowledge of the disease, however, dates from the report of Schenck²⁷ in 1898 and the later one of Hektoen and Perkins,²⁸ who named their fungus *S. schenckii*. Much of the present day knowledge of sporotrichosis is attributed to de Beurmann and Ramond,²⁹ of France, who in 1903 reported a case of sporotrichosis which was found by Matruchot and Ramond (1905) to be due to an organism which they named *Sporotrichum beurmanni*. In the following years de Beurmann and Gougerot and many other French workers established the identity of the disease in that country, while many reports appeared in the United States, Brazil, Argentina, Colombia, Germany, Austria, England, Turkey, Spain and Italy. These isolated reports were given greater attention when de Beurmann and Gougerot³⁰ published a collection of approximately 200 cases of sporotrichosis with a study of the disease and the organism.

26 Montagne, cited by Saccardo. *Sylloge fungorum omnium huiusque cognatorum*. Patavii, typis semmarii, 1886, vol 4, p 100.

27 Schenck, B. R. *Bull. Johns Hopkins Hosp.* 9: 286, 1898.

28 Hektoen, L., and Perkins, C. F. *J. Exper. Med.* 5: 77, 1900.

29 de Beurmann, L., and Ramond, L. *Ann. de dermat. et syph.* 4: 678, 1903.

30 de Beurmann, L., and Gougerot, H. *Les sporotrichoses*, Paris: Félix Alcan, 1912, p 852.

The first observation of radiation on cells of *Sporotrichum* in human tissue was made by Splendore³¹ in 1908, who termed the structure asteroid formation. The star-shaped bodies were found extracellularly in pus obtained from a verrucous, vegetative, hard and somewhat elastic lesion that had been present for twenty days on the right side of the face of an Italian woman living in São Paulo, Brazil³²

In 1908 Greco³³ described radiating forms in man and rat from Argentina. The following year, Harter and Gruyer,³⁴ in France, were able to demonstrate radiating forms of *Sporotrichum* in experimentally produced sporotrichosis in a guinea pig.

In 1934 Bordes, Berhouet and Errecart³⁵ published 4 cases of sporotrichosis from Uruguay. While examining hematoxylin and eosin-stained sections of a gummatous lesion in 1 of these cases, Talice noted the asteroid form described by Splendore. In 1935 Talice³⁶ published this case and an additional one in which radiating forms were shown. In 1939 MacKinnon³⁷ reported that he and Talice had found radiating sporotricha in the pus of 6 of 7 cases of sporotrichosis.

Moore and Ackerman¹ have recently observed the asteroid form of Splendore in sections of a lesion of gummatous sporotrichosis with lymphatic spread which occurred in a man aged 53 years, a native Missourian. This case is the first observed in the United States.

Pathology—Sporotrichosis is characterized by the production of various types of cutaneous and systemic lesions. These can be listed in brief as gummas, ulcers, funicle-like lesions, abscesses, nodules associated with lymphadenopathy and a general granulomatous response in skin, internal viscera or bony structures. The microscopic response varies according to the type of lesion present. In general, however, there is edema in the epidermal layers associated with irregular acanthosis, extensive or moderate, and at times suppuration, foci of which are particularly evident in the ulcerating type of lesion. There is a prominent infiltrate of polymorphonuclear leukocytes scattered throughout the pseudoepitheliomatous growth in the cutis, forming

31 Splendore, A. Rev. Soc. de sc., São Paulo 3 62, 1908.

32 Splendore, A. Brasil-med 23 361, 1909.

33 Greco, N. V. Rev. Dermat 1 78, 1908.

34 Harter, A., and Gruyer. Compt. rend. Soc. de biol. 61 309, 1909.

35 Bordes, C., Berhouet, A., and Errecart, L. M. Bol. Soc. med. quir. del Centro de la Republica 7 17, 1934.

36 Talice, R. V. Ann. de parasitol 13 576, 1935.

37 Talice, R. V., and MacKinnon, J. E., in Proceedings of the Third International Congress for Microbiology (1939), 1940, pp. 510-511.

microabscesses in some regions. In addition, the infiltrate in the cutis may consist of plasma cells, young connective tissue cells, many epithelioid cells, lymphocytes, some mast cells and giant cells of the Langhans type. The lymph spaces in the upper third of the cutis are usually dilated.

The granulomatous nature of the lesions is emphasized by the nodular formation. These nodules may be superficial or deep in the cutis. The center of the nodule usually shows necrotic masses or small abscesses, the chronic suppurative zone, and this area is surrounded by richly stained cells consisting of polymorphonuclear neutrophils, eosinophils, lymphocytes, red blood cells and macrophages. Closely adjacent to this area is the tuberculoid zone, which consists of many epithelioid cells and giant cells, varying in number, size and shape and often arranged in tubercle-like fashion. The peripheral, or outer, area of the nodule, the syphiloid zone, is made up of a rich cellular infiltrate of young connective tissue cells, lymphocytes, plasma and mast cells and an increased number of blood vessels, simulating a syphiloid appearance.

The nodules may be distinct and isolated, or several may merge to produce a large granulomatous mass. As a consequence of the granulomatous process, the elastic fibers may become irregular and broken. There may be a perivascular reaction, and the collagen bundles may be invaded by lymphocytes and plasma cells.

Fungus in Tissue—In tissue or pus the cells of *Sporotrichum* may be seen as short, blunt, rodlike or spindle-shaped forms, somewhat rectangular, basophilic, measuring 1 to 3 by 2 to 5 microns and occurring singly or in groups. Ovoid to spherical cells may also be seen, and all of these cells may be found, but with difficulty, either freely dispersed in the necrotic material or phagocytosed by mononuclear leukocytes or macrophages. These cells are gram positive and have a colorless, capsule-like periphery.

A second type, designated as the asteroid form, is characterized in tissue or pus by the presence of a radiate structure on the pathogenic fungus. The central cell may be spherical and thick walled (double contoured), it stains pinkish blue with methylene blue and eosin and measures approximately 5 microns in diameter (fig 3, 4 and 5). The rays vary in length from 2 to 8 microns and appear similar to those seen in actinomycosis. Acidophilic bodies may be seen throughout the nodule, which are made up of short rays. Other cells may have a mass of pink-staining material, which appears to be emanating directly from the cell wall. The radiating structures are observed within microabscesses surrounded by polymorphonuclear leukocytes and other cells of the inflammatory type.

MADUROMYCOSIS

Definition—Maduromycosis (mycetoma, Madura foot) is a chronic granulomatous, infectious process localized usually to the extremities but occasionally affecting other parts of the body. It is characterized by variously sized enlargements on the cutaneous surface, which eventually give rise to intercommunicating sinuses and fistulas, from which variously colored granules comprising fungous elements of the genera *Actinomyces* (*Nocardia*), *Madurella*, *Monosporium* (*Allescheria*), *Indiella*, *Trichosporium*, *Aleurisma*, *Torula*, *Phialophora*, *Glenospora*, *Aspergillus*, *Penicillium* and *Sterigmatocystis* can be obtained.

History—Maduromycosis probably dates back many years to the Sanskrit and the missionary writings referred to by Castellani and Chalmers³⁸. Most of the present day knowledge of the disease seems to have had its beginning in 1842, when Gill, from the city of Madura, in the Madras Presidency of India, described a condition of the foot which had deformed the extremity with fungoid excrescences, gave off a discharge and affected the joints, ligaments and cartilages. In 1846 Colebrook designated the disease "Madura foot". In 1845 Godfrey, a garrison surgeon in India, found some black granules in the tissues of an amputated foot with the infectious "ulcus grave," a disease which showed ulcers and sinuses with marked swelling. He had referred to this condition in a previous report as "morbus tuberculosis cutis". In 1848 Rustonji differentiated two clinical forms, one with a black substance and the other with yellow granules.

Carter,³⁹ after careful study, established the mycotic nature of the granules, recognized the black, yellow or white, and red types of granules and created the term "mycetoma". In 1886 he drew attention to the similarity between the granules of actinomycosis and those of mycetoma. Kanthack⁴⁰ in 1892 concluded that the yellow and black granules were of the same organism, *Actinomyces*, but Boyce and Surveyor⁴¹ established these as belonging to two distinct organisms. Their work resulted in the naming of the two disease entities, actinomycotic Madura foot and maduromycosis, as caused by other fungi. There have been a number of reports of cases since the work of these men, and Gammel⁴² in 1927 contributed a comprehensive and valuable review of the disease from the standpoint of etiology.

38 Castellani, A and Chalmers, A J. Manual of Tropical Medicine, ed 3, New York, William Wood & Company, 1920, p 2110

39 Carter, H V. Tr M & Phys Soc Bombay 6 104, 1861, 7 206, 1862

40 Kanthack A A. J Path & Bact 1 149, 1892

41 Boyce R, and Surveyor, N F. Phil Tr, London 185 1, 1894

42 Gammel, I A. Arch Dermat & Syph 15 241, 1927

Pathology—Grossly, maduromycosis has the appearance of a large tumefaction of varying size and shape. The cutaneous surface shows scarring with ulcerating nodules and fistulas over the enlarged swelling.

The microscopic picture of mycetoma is distinctly granulomatous with the general picture of active proliferation and infiltration of the various types of cells encountered in other mycotic granulomas. There is local necrosis and abscess formation, followed by the production of fibrous tissue and ultimately scar formation. Some of the abscesses suppurate, while others tend to sclerose. The abscesses may be small and round, or they may coalesce and become large and irregular or elongate. The granules are found within these abscesses, which eventually develop into sinuses and often reach the surface of the skin. The subsequent histopathologic character of the lesions is dependent on the genus and the species of fungus involved. The actinomycotic and aspergillotic lesions have been described. Lesions due to *Penicillium*, *Glenospora* and *Steinmatocystis* are essentially similar to those produced by *Aspergillus*. Cases of mycetoma caused by *Madurella* and to a certain degree by *Torula* (*Phialophora jeanselmei* as described by Symmers and Sporer⁴³) are essentially similar. In these cases the suppurating abscess is made up of small and large lymphocytes, large mononuclear and polymorphonuclear leukocytes, red blood cells, cellular detritus and albuminoid bodies, all in and around the granules. Some of the small abscesses are made up predominately of lymphocytes and plasma cells, while others contain multinucleated giant cells, surrounded by a thick, sclerotic wall. Plasma cells may be seen in abundance scattered throughout the lesion. The wall of the abscess is made up first with a layer of connective tissue fibrils or large mononucleated cells, which are more or less spheroidal and vacuolated (foam cells), owing to the presence of fats. Surrounding this are a second layer comprising granulation tissue, well vascularized, and then an outer layer of dense connective tissue. Eosinophils have been observed occasionally. Lesions caused by *Monosporium* (*Allescheria*) *apiospermum* seem to lack the fatty histiocytes.

Fungus in Tissue—In the lesions the fungi causing maduromycosis or mycetoma are seen in the form of grains or granules varying in size, shape and color, depending on the organism responsible for the disease. The granules in the past have been classified according to color. This method is not accurate botanically, since different-colored granules may, on occasion, give rise to similar

43 Symmers, D., and Sporer, A. Arch Path 37:309, 1944

organisms. However, from a pathologist's standpoint such a classification may have its merits. Actinomycotic granules may be black red or yellowish green. Other fungi producing maduromycosis may be listed according to the color of their granules as follows: (1) black granules—*Madurella*, *Glenospora*, *Torula* or *Phialophora*, *Aspergillus* and *Penicillium*, (2) white or yellowish white granules—*Monosporium* (*Allescheria*), *Indiella*, *Sterigmatocystis*, *Cephalosporium* and *Acremonium*, (3) greenish yellow granules—*Aspergillus*, (4) red granules—*Aspergillus* and *Rubromadurella*.

The nature and the types of actinomycotic granules have been described under the heading "Actinomycosis." The grains of maduromycosis vary according to the fungus and the age of the granule itself. The young granules are in general composed of intertwining septate hyphae and occasionally spherical to ovoid cells, the chlamydospores. Mature granules are made up of a variable number of distinct zones. The innermost zone is made up of mycelial elements, hyphae, chlamydospores, pigmentary granules and disintegrated leukocytic granules. This is surrounded by a deeply pigmented, irregularly amorphous zone. Closely adjacent to this area may be seen radiating hyphae and chlamydospores. Dispersed through the granule are leukocytes in various stages of degeneration. In addition to these usually characteristic zones there may be seen an outer fringe or zone on some granules which is acidophilic and has fine filamentous prolongations, more or less radiate and refractile, or it may consist of a narrow, pink-stained rim, or it may be composed of clubs as seen with *Actinomyces*. This is the zone of radiation or radiate formation.

Radiate formation on *Actinomyces* producing mycetoma is fairly common. This same phenomenon is likewise observed not infrequently on other organisms producing maduromycosis. No attempt has been made to cover completely the literature dealing with this disease in order to find the number of cases in which acidophilic material was found on the outer region of the involved granules. The descriptions of granules and the observations reported in some of the papers read however, seem to indicate that its occurrence is not rare.

Radiate formation on *Aspergillus* in maduromycosis has been observed by da Fonseca. The granules were greenish yellow. Various species of *Madurella* when seen in the tissue of mycetoma have likewise revealed radiate formation. De Almeida⁴⁴ in 1932 illustrated various granules seen in maduromycosis, several of which were probably *Madurella*. In one (his fig 1) he described a thickened and irregular peripheral coating which stained pink with eosin. This no

doubt was radiate formation. In 1926 Gammel, Miskdjian and Thatcher⁴⁵ described mycetoma occurring in a 26 year old Mexican born in Texas, which was due to *Madurella americana*. "The outer zone was a small rim which had a pink tinge." This, too, was radiate formation. In 1938 Hanan and Zurett⁴⁶ published observations on mycetoma developing in a white man as a result of wood splinters entering his foot. The black granules in the tissue were formed by the fungus *Madurella lackawanna*. These authors described four zones in all granules and a fifth zone in some, which was "composed of clubs such as are frequently observed on the periphery of actinomycotic granules."

In 1935 Talice⁴⁷ described the red granules of a case of maduromycosis caused by *Rubromadurella langeroni*. There was a definite zone of radiation, which was both well described and figured as the acidophilic substance of radiate formation. In 1941 Niño⁴⁸ published a case of maduromycosis caused by *Monosporium apiospermum*, in which the yellowish white granules had peripheral structures simulating clubs. Lesions of maduromycosis due to *M. apiospermum* have been observed in this country with an acidophilic substance on the peripheral structures of the granules which could be considered as radiate formation. Of particular interest is the recent description published by Symmers and Sporer of a mycetoma of the hand which showed black granules. The organism was later identified by Emmons as *Phialophora* (*Torula*) *jeanselmei*. The authors described the granules, in part, as follows, "In some instances clumps of degenerate chlamydospores merge imperceptibly into broad bases composed of acidophilic material, projecting from the border of which are pinkish-staining needle-like formations representing, probably, mycelial elements." A section of the tissue was examined and studied through the courtesy of Dr. Symmers, and it was apparent that the acidophilic material was identical with radiate formation (fig 4, 1). In a later publication dealing with maduromycosis as experimentally reproduced in rabbits with granules from the human case of mycetoma, Symmers⁴⁹ was able to demonstrate, fifty-eight days after inoculation, granulomatous lesions with the formation of granules. The younger granules showed the same acidophilic material in the form of projections from the granular mass.

45 Gammel, J. A., Miskdjian, R., and Thatcher, H. S. *Arch. Dermat. & Syph.* **13**: 66, 1926.

46 Hanan, E. B., and Zurett, S. *Arch. Dermat. & Syph.* **37**: 947, 1938.

47 Talice, R. V. *Ann. de parasitol.* **13**: 584, 1935.

48 Niño, F. L. *Bol. d. Inst. clin. quir.*, 1941, p. 483.

49 Symmers, D. *Arch. Path.* **39**: 358, 1945.

It is obvious that most of the genera producing mycetoma are capable of producing granules which give evidence of radiate formation or show acidophilic material at the periphery

RARE RADIATE FORMATION

There are three other mycoses in which radiate formation has been seen on the causative fungi and which may be considered along with those already described paracoccidioidal granuloma, chromomycosis and North American blastomycosis. The diseases in which these radiations are found are not uncommon, and it is felt that with careful study more instances of the acidophilic material may very well be described

PARACOCCHIDIOIDAL GRANULOMA

Definition—Paracoccidioidal granuloma, or South American blastomycosis, or Lutz–Splendore–de Almeida disease, is an acute or chronic granulomatous infection, localized or generalized, involving skin, mucous membranes, lymphatics, internal viscera and bony structures

History—The disease was first described by Lutz⁵⁰ in 1908, when he considered it a pseudococcidioidal granuloma with the organisms occurring chiefly in giant cells. The fungus was isolated in pure culture from the lymph nodes and salivary glands of the patient. In the same year Carini⁵¹ described a case with primary lesion of the buccal mucosa. In 1909 Splendore described a case of generalized blastomycosis, thus calling attention to two clinical types, the localized, buccal mucosa type and the generalized. He expressed the belief that the two types are caused by two different fungi. Several reports were written by Splendore,⁵² and in 1912 he established the specific name of *brasiliense* for the organism and placed it in the genus *Zymonema* of de Beurmann and Gougerot⁵³. The following year⁵⁴ a more lengthy description of the disease was published. Since that time the disease and the organisms have been well described by several investigators, including de Almeida, da Fonseca, Niño, Mazza, Moore and others.

Reference to radiate formation on cells of *Paracoccidioides* was perhaps first made by Weidman in 1932, when he cited a paper pub-

50 Lutz, A. *Brasil-med* 22 121 and 141, 1908

51 Carini, A. *Rev Soc de sc, São Paulo* 3 120, 1908

52 Splendore, A. *Bull Soc path exot* 5 313, 1912

53 de Beurmann, L., and Gougerot, H. *Bull et mém Soc méd d hôp de Paris* 28 222, 1909

54 Splendore, A., in *Onore del Prof. Angelo Celli nel 25 anno di insegnamento*, Turin, 1913, p. 421

lished by de Almeida in 1929. Obviously this was a mistake, since what was considered to be denticulate or radiate extensions on this organism have been proved to be multiple gemmation or budding. After Weidman, de Almeida,⁵⁵ in his publication of 1934, studied 2 cases of paracoccidioidal granuloma, one of the lung and the other of the skin. In the sections of tissue from these cases de Almeida described what he believed to be true radiate formation.

Pathology—From the point of view of pathology, paracoccidioidal granuloma, or South American blastomycosis, in many respects mimics North American blastomycosis, or Gilchrist's disease. There is a multiplicity of clinical types and forms with corresponding changes in the histologic response.⁵⁶ Grossly there can be seen ulcers, vegetative formations, pustules, papules and tuberculoid and syphiloid manifestations. In systemic disease the lesions are chiefly ulcers, originating mostly in the lymphoid tissue as a result of the lymphatic spread of the organism. The hepatic, the splenic and the uncommon pulmonary lesions are seen generally as nodules and presumably are due to the hematogenous spread of the fungi. Microscopically there is a granulomatous response, such as can be seen in other mycotic granulomas, with an infiltrate of lymphocytes, plasma cells, polymorphonuclear leukocytes (many forming abscesses) and giant cells (many phagocytosing organisms), the last often arranged in tubercle-like fashion.

Fungus in Tissue—In tissue or pus *Paracoccidioides* is seen as a spherical or ovoid cell varying from 1 to 30 microns in diameter, with a thick wall. The larger cells show simple or multiple budding in the form of minute spherical, ovoid or bacillary gemmules. These buds are arranged on the outer wall of the mother cell and when seen in cut sections appear peripherally and radially arranged to simulate radiate formation. On the other hand, de Almeida observed radiations on the cell wall which gave the appearance of a thickened, irregularly denticulated wall.

An examination of a number of slides of both the cutaneous and the visceral lesions of paracoccidioidal granuloma was finally rewarded by the finding of what could be interpreted as radiate formation. A peculiar phenomenon of *Paracoccidioides* in tissue seems to be its ability frequently to produce an area of lysis about itself. This area apparently is one of liquefaction and probably results from the lytic action of an enzyme liberated by the fungus in its growth. In paraffin sections the area appears as a clear space. In some of these spaces on the wall of the fungus in hematoxylin and eosin-stained sections one

55 de Almeida, F. P. Ann. Fac. de med. da Univ. de S. Paulo **10** 163 1934.

56 Moore, M. J. Invest. Dermat. **6** 149, 1945.

can see pink-staining extensions in the form of fine prickles or in that of irregular short or elongated formations. These structures, which were considered to be radiations, were seen both on a single cell and on the multiple budding cells (fig 4, 2 and 3)

In examining tissue for radiating structures of *Paracoccidioides*, there is a phenomenon which may confuse the observer. The fungus is often phagocytosed by giant cells and by large macrophages. The latter give rise to what has been termed *pseudococcidioides*, which has been observed in pus rather frequently,⁵⁷ especially when the fungus involved was *Paracoccidioides cerebriformis*. When one of these macrophages becomes disintegrated and the fungus set free, bits of the macrophage may adhere to the fungous cells. These particles of clinging macrophage stain with eosin and may resemble radiate formation

CHROMOMYCOSIS

Definition—Chromomycosis (chromoblastomycosis, dermatitis verrucosa) is a chronic granulomatous disease affecting the skin chiefly, with rare involvement of regional lymph nodes. The lesions are commonly found on the extremities and may be papular, nodular, verrucous or papillomatous, with or without ulceration and abscess formation.

History—The discovery of chromomycosis was made by Pedroso in 1911. Reporting from Brazil, he described a "black blastomycosis" due to dark brown cells which were seen in sections of the diseased tissue. In 1920 Pedroso and Gomes⁵⁸ published this case with 3 others from Brazil and named the fungus *Phialophora verrucosa* on the basis of a case reported in 1915 by Lane⁵⁹ and also by Medlar,⁶⁰ the organism in that case having been described as *P. verrucosa* by Thaxter. Since 1920 numerous cases have been reported from many parts of the world. These have been reviewed by de Almeida,⁶¹ Moore and Mapother⁶² and Weidman and Rosenthal⁶³. In 1942 Pardo-Castello, Leon and Trespalacios⁶⁴ reported 31 cases from Cuba and included a fine description of the clinical types of chromomycosis.

57 Moore, M. Arch Dermat & Syph **38** 163, 1938

58 Pedroso, A., and Gomes, J. M. Ann paulist. de med e cir **11** 53, 1930

59 Lane, C. G. J Cutan Dis **33** 840, 1915

60 Medlar, E. M. Mycologia **7** 200, 1915

61 de Almeida, F. P. Mycologia medica. Estudo das mycoses humanas e de seus cogumelos, São Paulo, Companhia Melhoramentos, 1930, p. 583

62 Moore, M., and Mapother, P. Arch Dermat & Syph **41** 42, 1940

63 Weidman, F. D., and Rosenthal, L. H. Arch Dermat & Syph **43** 62, 1941

64 Pardo-Castello, V., Rio Leon, E., and Trespalacios, F. Arch Dermat & Syph **45** 19, 1942

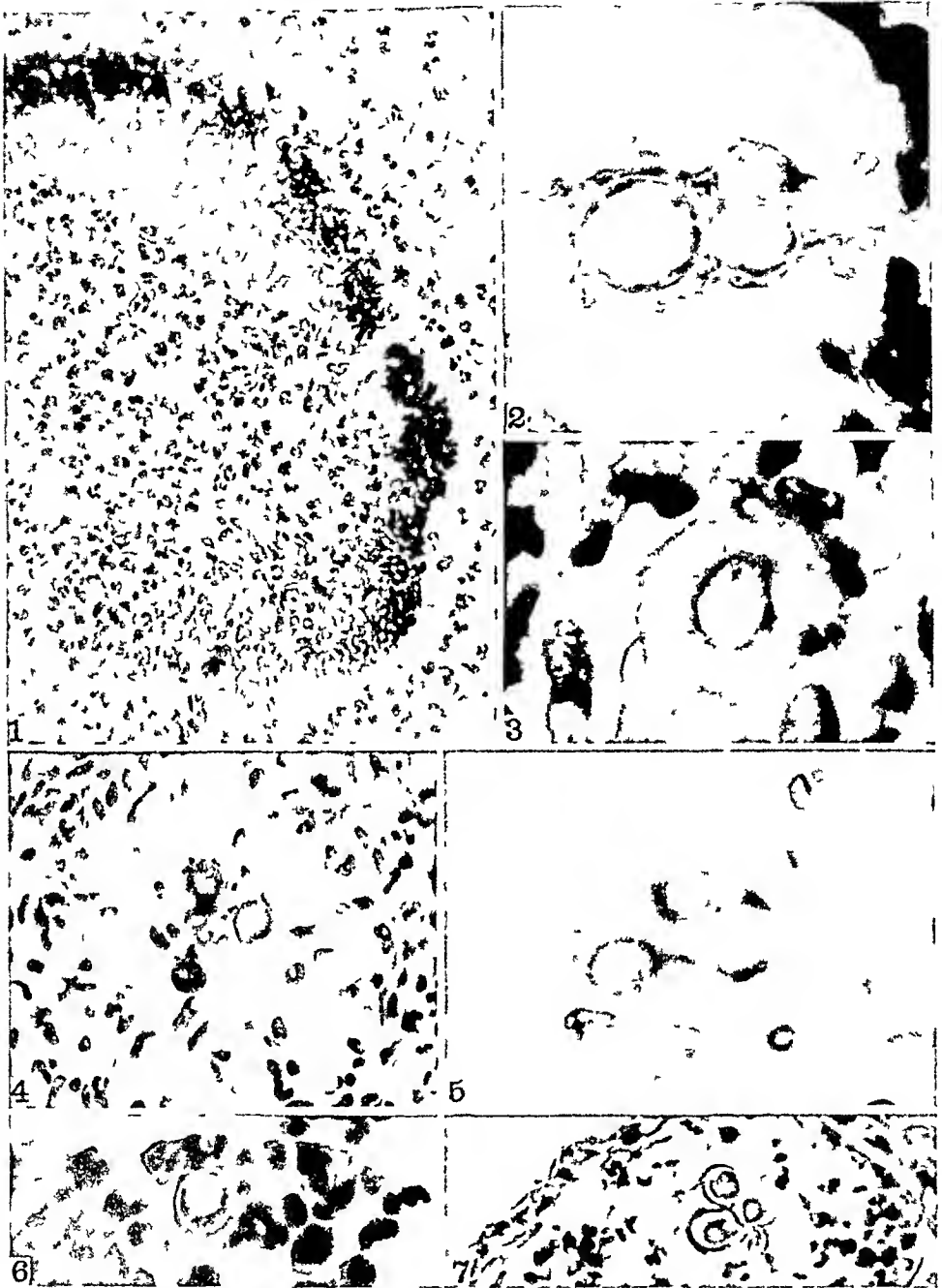


Fig 4—1, Section of granule of *Phialophora* (*Torula*) *jeanseinei* showing fringe of acidophilic material, case of Drs Symmers and Sporer, hematoxylin and eosin, $\times 361$ 2 *Paracoccidioides brasiliensis* with multiple budding and acidophilic material, hematoxylin and eosin, $\times 1,110$ 3 *P. brasiliensis* showing acidophilic radiate-like substance in tissue, hematoxylin and eosin, $\times 1,110$ 4 Fungous cells of chromomycosis with acidophilic incrustations in tissue, hematoxylin and eosin, $\times 425$ 5 Fungous cells of chromomycosis with irregular peripheral radiations in tissue, hematoxylin and eosin, $\times 1,110$ 6 Fungous cell of blastomycosis with acidophilic substance partially involving the wall, seen in a pulmonary nodule, hematoxylin and eosin, $\times 648$ 7 Fungi of blastomycosis showing adherent acidophilic material on cell walls, seen in a cutaneous abscess, hematoxylin and eosin, $\times 425$

In 1943 Moore, Cooper and Weiss⁶⁵ reviewed the cases reported in the United States

The first report of radiate formation on cells of fungi producing chromomycosis was made by de Almeida in 1934. De Almeida observed the radiate structures in tissue furnished him by Maciel from a case which occurred in Santos, Brazil, in 1916. The second case was that of a patient in a hospital in São Paulo, Brazil. In addition to these 2 cases, the diagnosis of which I have confirmed, there has been a third case reported from Brazil in which the characteristic radiations were present on the cells.

Pathology—Grossly, chromomycosis manifests itself by the production of various types of lesions of the skin, chiefly of the extremities but also of the face, the ear, the neck, the chest, the shoulders and the buttocks. On the basis of the cases reported in the literature and 31 cases observed in Cuba, Pardo-Castello and his associates have classified chromomycosis into five types: the verrucous or papillomatous, the tuberculoid, the syphiloid, the psoriasiform and the elephantiasic. Briefly these may be described as follows: 1. The verrucous or papillomatous type exhibits first papules and then nodules, which enlarge to show eventually abscesses, rarely suppuration, and finally central healing and scar formation. 2. The tuberculoid type shows small patches or nodules with erythematous areolas. 3. The syphiloid form in the early stages is nodular and scaly, with some erythema, and then becomes ulcerated and covered with crusts. The nodules are small, in the early lesions, and are flattened, serpiginous, annular or arcuate in their arrangement. 4. The psoriasiform type is characterized by superficial inflammatory lesions with infiltration, covered with thick, adherent white scales. 5. In the elephantiasic form the extremities are greatly enlarged, and there are many features characteristic of the other types with the addition of cicatrization.

Microscopically, the lesions of chromomycosis have a number of features in common with other mycotic granulomas. The epidermis is hyperplastic, showing hyperkeratosis and acanthosis. Within the elongated rete pegs may be seen microscopic abscesses filled with polymorphonuclear leukocytes, cellular debris and fungi. Within these areas may also be seen Langhans giant cells phagocytosing fungi.

The dermis responds most strongly to the organism. There are edema, pronounced cellular infiltration and in older lesions evidence of fibrosis. The infiltrate consists of polymorphonuclear leukocytes, lymphocytes, plasma and epithelioid cells, eosinophils, Russell's fuchsin bodies, macrophages and giant cells of the foreign body or Langhans

65 Moore, M., Cooper, Z. K., and Weiss, R. S. J. A. M. A. 122: 1237, 1943

type, the last sometimes arranged in tubercle-like fashion. In the early lesions there is an extensive infiltration in which cells of the types just mentioned participate, accompanied by thickening, edema, hyperplasia, hyperkeratosis and acanthosis. Granulomatous changes are not apparent, and fibrosis, which is most noticeable in older lesions, is lacking. Scattered throughout the rich cellular infiltrate, chiefly polymorphonuclear leukocytes, can be seen the thick-walled cells of the fungus. The older lesions show some necrosis and abscess formation, but these are not as pronounced as in Gilchrist's disease. The fungus can be seen also in the giant cells and in the abscesses.

Fungus in Tissue—In tissue or pus the large sclerotic cells of the fungus are dark brown, thick walled and spherical or irregular in outline, they may be single, multiple or multilocular and are approximately 3 to 10 microns in diameter. They reproduce by enlargement and cross wall development to form mulberry-like clusters, but never by budding. In old, necrotic lesions there may be seen short filaments, which are germinations of the spherical, sclerotic cells.

Radiate formation on the dark brown, thick-walled cells, as on the cells of *Paracoccidioides*, is seen as an eosinophilic or acidophilic corona on the wall of the fungus (fig 4, 4 and 5). The acidophilic material, however, is not uniform in appearance, being thicker on some parts of the cells and lacking on other parts. The projections may extend outward either as an irregular mass or as somewhat rounded elongations, varying in length. From their appearance staining quality and location, they should be considered as radiate formation.

NORTH AMERICAN BLASTOMYCOSIS, OR GILCHRIST'S DISEASE

Definition—Blastomycosis is a granulomatous, infectious process which is protean in its manifestations and caused by budding thick-walled, yeastlike organisms.

History—The first case of blastomycosis was reported by Gilchrist in 1894 at the session of the American Dermatological Association. He found yeastlike bodies in lesions described by the attending physician as scrofuloderma. Two years later⁶⁶ this case was published in detail. In 1896 Curtis⁶⁷ reported a similar organism from a myxomatous tumor of the leg. In the same year, Gilchrist and Stokes⁶⁸ published a short paper on a second case of blastomycosis, and this was published

66 Gilchrist, T. C. *Johns Hopkins Hosp. Rep.* **1** 269, 1896.

67 Curtis, F. *Ann. Inst. Pasteur* **10** 449, 1896.

68 Gilchrist, T. C., and Stokes, W. R. *Bull. Johns Hopkins Hosp.* **7** 129, 1896.

in detail in 1898⁶⁹ The observations of Hyde and Montgomery on the clinical, pathologic and mycologic aspects of the cutaneous lesions of the disease were published by Montgomery⁷⁰ in 1902 in an article which is a masterpiece

Pathology—Blastomycosis closely resembles tuberculosis, a neoplasm or syphilis In the skin are seen pustules, ulcerations, nodules, gummas and papillomas, granulomatous in nature In the internal organs the disease manifests itself as miliary or large-sized nodules, abscesses and neoplasm-like formations

Microscopically, the epithelium is irregular, thickened and elevated in parts and thin and depressed in others The stratum corneum may be lacking in some places and hyperkeratotic in others The epidermis is hyperplastic, with long extensions into the corium Many of the pseudoepitheliomatous proliferations contain the miliary abscesses characteristic of the disease The abscesses are widespread throughout the epithelium, vary in size and number and are made up of epithelial detritus, leukocytes (some in various stages of degeneration), epithelial cells, nuclear fragments, red blood cells, giant cells of the Langhans type and budding, yeastlike cells of the fungus The abscesses are surrounded by flattened, apparently functionless epithelial cells, which form a type of nest The rete is usually edematous and infiltrated by leukocytes Cornified cells may be seen as isolated forms, in groups or in whorls, and the giant cells, occasionally surrounded by a few leukocytes, may be seen in the dermis, occurring singly or arranged in tuberculoid fashion

In the corium one may find the type of abscesses seen in the epithelium There are inflammatory changes, which may be subacute, acute or chronic The infiltrate, which may be perivascular, with the vessels hyperplastic, is made up of leukocytes, plasma cells and young connective tissue cells, varying in density Mast cells and giant cells may also be seen Plasma cells, giant cells and new connective tissue cells may occasionally show hyaline degeneration, and densely infiltrated areas show complete destruction of the collagen Tubercle-like formation may also be seen

Fungus in Tissue—In tissue the fungus is seen usually in the miliary abscesses, in the epithelium and in the corium, always surrounded by an inflammatory process The number of cells of the fungus is not constant and varies in these locations Occasionally, an area with many giant cells and abscesses may show one or two fungous cells, whereas other areas with few abscesses or giant cells

69 Gilchrist, T. C., and Stokes, W. R. J. Exper. Med. 3: 53, 1898

70 Montgomery, F. H. J. A. M. A. 38: 1486, 1902

may show many budding, yeastlike organisms. The fungus is seen as single or budding, yeastlike cells, thick walled, having a double-contoured appearance and measuring approximately 5 to 12 microns in diameter. In old, necrotic lesions one may see simple branching cells, which may attain a length of 20 microns.

As in the case of chromomycosis and that of South American blastomycosis, eosinophilic or acidophilic material may be rarely seen on the walls of the fungous cells. In the few observations of this type of formation, the pink-staining substance was noted only on a portion of the cell wall as an irregular projection which extended a short distance from the wall (fig 4, 6 and 7). The substance did not have the size of that noted on fungi of chromomycosis and paracoccidioidal granuloma.

OTHER RADIATE FORMS

In addition to the diseases mentioned, there are others in which radiation on the infecting organism has been described. On rare occasions the radiate forms have been noted, chiefly in animals but also in man, on such organisms as *Mycobacterium tuberculosis* and on *Staphylococcus* as seen in the disease botryomycosis. In 1919 Magrou,⁷¹ while discussing the actinomycetoid form of botryomycotic granules, presented evidence of radiation occurring on *Monilia albicans* in an experimentally infected rabbit. The organism had been obtained from human sputum. Radiate forms were observed in the kidney, where numerous inflammatory foci were present. Radiate forms of *M. (Candida) albicans* in man have not been reported in the literature. The organism of actinobacillosis in animals has also shown the radiation effect. Ravaut and Pinoy⁷² have demonstrated this phenomenon on *Actinobacillus* in man.

The presentation of radiation effects on fungi in tissue would perhaps be incomplete without a discussion of other phenomena, chiefly gloea and capsule formation, but also granular projections, occurring on fungous cells in tissue.

Gloea Formation—The production of gloea is perhaps more common with bacteria. Fungi in the pathogenic state affecting man can form gloea in a limited number of instances. This is evident in the hair diseases known as piedra (piedra nostras, trichosporosis), both the Brazilian and the Colombian type (fig 5, 1), and lepothrix (trichomycosis axillaris) (fig 5, 2). The fungi grow and surround the hair in the form of nodules or masses, the elements of which seem to be held together by a mucilaginous or gelatinous matrix, the gloea. In cultures

71 Magrou, J. Ann de l'Inst Pasteur **33** 344, 1919.

72 Ravaut, P., and Pinoy, F. Ann de dermat et syph **10** 417, 1909.

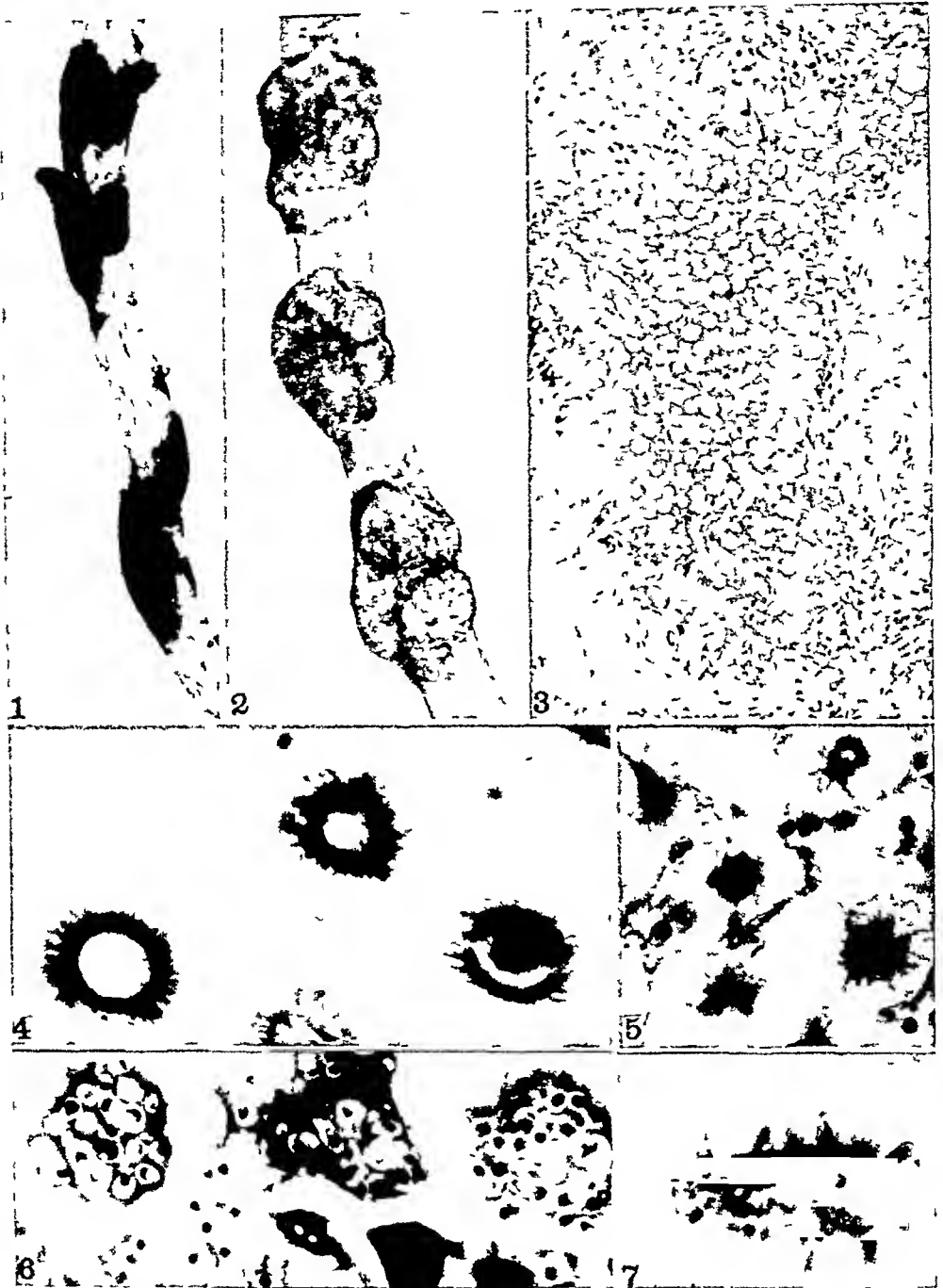


Fig 5—1 Hair with nodules of piedra, $\times 53$ The gloea has hardened to form pebble-like concretions 2 Nodules of leprothrix on a hair, $\times 74$ The gloea remains mucoid, and the granule consequently is soft 3 Cyst-like lesion of the brain showing mucoid encapsulated cells of *Cryptococcus* forming a jelly-like mass, hematoxylin and eosin $\times 795$ 4 Cells of *Cryptococcus* showing radiating extensions of the cell walls, hematoxylin and eosin, $\times 956$ 5 Irregular cell wall extensions of *Cryptococcus*, hematoxylin and eosin, $\times 3985$ 6 *Histoplasma* capsulatum in reticuloendothelial histiocytes with clear mucoid capsules hematoxylin and eosin, $\times 919$ 7 Pearform cell of *Histoplasma* grown on agar with the characteristic cell wall extensions, iron alum-hematoxylin stain, $\times 1,222$

on artificial mediums, certain fungi are capable of producing a gloea-like substance, which is liberated with spores from an endogenous spore-forming organ and serves to hold the spores together until they reach full maturity. The gloea disintegrates, and the spores are set free. Among pathogenic fungi a notable example of this phenomenon can be found with *P. verrucosa*, an etiologic agent of chromomycosis.

Capsule Formation—In contrast to gloea, which holds the fungus elements together *en masse*, the capsule is a mucoid, mucilaginous or gelatinous substance surrounding the individual cell. The capsules do not stain with the ordinary dyes. When seen in fungous groups or masses, the capsular material may appear as gloea, and the whole may resemble a jelly-like, gelatinous or mucoid cyst (fig 5, 3). An outstanding example of this is seen in lesions of cryptococcosis (torulosis), especially in those involving the brain. The causative organism is *Cryptococcus hominis* (*Cryptococcus neoformans*).

Of particular interest with regard to *C. hominis* is the fact that on occasion one can see encapsulated forms with radiations extending from the cell wall into the capsule, usually to the edge of the mucoid substance (fig 5, 4 and 5). This type of radiation, however, is not identical with that described for such organisms as *Actinomyces*, *Coccidioides* or *Sporotrichum*, since the rays are definitely continuous with, or are a part of, the cell and stain identically.

Another well known example of capsule formation is that seen on cells of *Histoplasma capsulatum* in tissue. The capsules vary in thickness and, like those of *C. hominis*, do not stain ordinarily (fig 5, 6). In 1940 Henri⁷³ suggested the possibility of radiation or actinomycetoid formation with *H. capsulatum*. It is probable that he was referring to the tuberculate condition of the large spherical to pyriform cells of *Histoplasma* in culture (fig 5, 7). These structures are functionless tubelike projections which are part of, and extend from, the cell wall. They resemble radiate formation and should not be confused with the acidophilic material.

Capsules may also be seen, under varying conditions and in various organs, on such yeastlike organisms as *M. (Candida) albicans*, *Zymonema* (*Blastomyces*) *dermatitidis*, *Paracoccidioides brasiliensis* and *P. cerebriformis* and others. In fact, capsule formation is not limited to tissue but may be induced under certain conditions of cultural growth. Huge capsules were formed on cells of *P. cerebriformis* when the organism was grown on wort agar.⁵⁷

73 Henri, A. T. J. Bact. **39** 113, 1940

Cell Wall Extensions—Many fungi when grown on artificial mediums produce various types of spores, such as ascospores, conidia, macroconidia or fuseaux, chlamydospores and others, which may have as a characteristic echinulate, granular or radiating projections developing from the spore wall. Spores of species of the genera *Aspergillus*, *Scopulariopsis*, *Ustilago* and others show small spines and are said to be echinulate. Although characteristically seen in cultures, as in the case of the first two genera, and in the parasitic phase on plants, as in the case of *Ustilago*, such spiny spores may be seen in parasitized human tissue. This is simply because the fungi in this case may be considered as secondary invaders and perhaps live as saprophytes in the tissue. Although producing disease in man, the fungi are not capable of adapting themselves to a parasitic form, such as is found with many of the pathogenic fungi. The fuseaux or macrospores of *Microsporum*, *Trichophyton* and *Epidermophyton*, the so-called dermatophytes, may show granular formations. These spores, however, are limited to cultures. Various organisms of the *Dematiaceae*, as well as some of the *Phycomycetes*, may show in culture, on the spores and the filaments, granular projections or incrustations.

COMMENT

Radiate formation on fungi in tissue is not a species-specific or genus-specific characteristic or phenomenon. Such fungi, referred to as actinophytes by Lignières and Spitz,⁷⁴ may include, as indicated by Magrou, many genera and species. Except for such organisms as *A. bovis* or *israeli*, and perhaps *C. immitis*, with which radiate formation is commonly associated, this structure in human tissue is sufficiently rare to be given further consideration here. It is worthy of note also that radiations do not always occur on *Actinomyces* and *Coccidioides*, for one may study tissue from many cases and find, first, that not all the sections will exhibit radiation forms and, secondly, that in the same sections some organisms will be actinomycetoid while others will lack the acidophilic substance. This inconstancy is of course greatly accentuated in the other diseases listed in this paper in which the radiation effect is not common. The finding of radiations with so many varied organisms would indicate that this formation is a concomitant finding frequently associated with the organisms in tissue.

The type and the form of the acidophilic substance have led to a great deal of speculation as to the nature and the source of this material.

⁷⁴ Lignières, J., and Spitz, G. *Rev. Soc. med. argent.* **10** 5, 1902, *Arch. de parasitol.* **7** 428, 1903.

A brief review of the types of radiation should be interesting. In the case of actinomycosis, reference is generally made to the club formation. This means that the terminal portion of the radiating structure is broader than that part which is closer to the fungous cell or mass. In some cases, not all, it has been possible to demonstrate filaments of *Actinomyces* within these rays, and Weidman has described the structure in these cases as follows, "the hyaloid incrustment being continued like a coat of ice over the surface of the colony and its projecting terminal hyphae." In some granules of *Actinomyces* the acidophilic substance seems to be in the form of irregular projections, lobulated masses and unequally thickened coverings. In the case of aspergillosis the radiations do not usually appear club shaped, but rather take the form of elongated crystals, with short, angular projections and with "broken-off" or abrupt tips. They may also be somewhat cylindric, as described by Wiedman, uniform or irregular in size, appearing at times like a mass of crystalline needles. There is in addition another type which appears to be in the form of an emanating substance on the hyphae and which has been compared to the Hülle cell seen on aborted reproductive structures of *Aspergillus*. On *C. immitis* may be seen prickles of uniform size and appearance extending from the wall of the mother cell, irregular radiating masses, and even extremely long filiform or tubular rays, as described by de Almeida. Club formation may also be seen on occasion. On cells of *S. schenckii* the rays take on an asteroid or a star-shaped arrangement, are somewhat tubular in appearance and may be of equal or unequal length. In the case of maduromycosis the radiation may be of the type seen on *Aspergillus* or of the actinomycetoid form. As regards paracoccidioidal granuloma, chromomycosis and blastomycosis, the radiation effect is seen as an acidophilic substance of non-uniform thickness either completely or incompletely involving the cell wall of the fungus.

It is apparent that the form of the radiations varies considerably with the organism and with the disease. An explanation for this may perhaps be found in the type of pathologic condition that the fungus produces. All of the diseases listed have in common leukocytic infiltration of a variable degree, inflammation of a varying degree and a terminal granulomatous response, or, as preferred by some, a chronic, progressive inflammatory response. In some diseases, particularly actinomycosis, maduromycosis and to a lesser degree coccidioidal granuloma, there is evidence of marked suppuration with sinuses being formed and pus constantly flowing to either the surface of the body or to adjacent tissues. In aspergillosis suppuration and large abscess or even cavity formation are present, but the flow of pus is not of the

rapidly spreading type seen in actinomycosis. The process may be compared with a stagnant pool which increases in size simply by erosion and disintegration of its surroundings. In sporotrichosis, blastomycosis, paracoccidioidal granuloma and chromomycosis, suppuration may be noted, but here too the flow of the pyogenic debris is slow and again results in abscesses of varying size. Sinuses are rare in these diseases. The variation in the type of rays may, therefore, depend on the motility of the fluid surrounding the organism. It is known that a constantly flowing fluid will tend to round up the tips exposed to the current and that, on the other hand, crystals with sharp edges and needle-like formations will develop in a quiescent fluid. Such an explanation may seem plausible for actinomycotic and aspergillotic granules, but the appearance of the small amount of the incrusting substance or radiate formation on the organisms of chromomycosis, paracoccidioidal granuloma and blastomycosis would be difficult to explain on this basis alone.

The nature and the source of the rays present perhaps the most intriguing problem. Unfortunately, the amount of experimental work done toward solving this phase has been limited by the amount of available material. With the knowledge that these structures can be produced regularly in animals, however, the amount of material can be greatly enlarged, so that an answer should be forthcoming before long. Be that as it may, the theories advanced as to the nature and the source of the radiations have been many and confusing.

Lichtheim in 1882 suggested that the rays were aborted productions of the fungus. Boyce in 1893 used various stains on the aspergillotic granules and concluded that the rays were distinct cell elements. Renon in 1897 agreed with Lichtheim that they were aborted fungous productions but also pointed out that the granules represented the index of the extreme defense of the body and the lowered resistance of the fungus. In other words, this radiation phenomenon could be interpreted to mean either a defense mechanism set up by the fungus or an offense set up by the body. Lignieres and Spitz, supported by Brumpt, regarded the radiate formation as "young protoplasm, capable of budding and serving as nutrient for the filaments in the interior of the grain." On the other hand, Ravaut and Pinoy considered the actinomycotic masses as the result of a mixed production depending on parasite and host. They compared the formation of masses to that of the hold-fasts of certain fungi parasitic on plants (Peronosporaceae).

Pinoy⁷⁵ expressed the belief that the radiate formation is a result of the digestive action of the host on the membrane of the fungus.

75 Pinoy, E. Bull Inst Pasteur **11** 929 and 977, 1913

Magrou felt that the actinomycotic form resulted from the parasitic life of the organism—a defensive reaction of the leukocytes and of the humors which accumulate around the parasite. In other words, the radiate forms have attained a “state of symbiosis with the leukocytes of the vertebrates”

Bayne-Jones⁷⁶ in 1925 presented the two theories advanced as to the nature of clubs. One attributed to the rays a developmental part in the life cycle of organism (theories of Boyce and, especially, of Lignieres and Spitz, supported by Brumpt). The other theory regarded the club as a thickening of the sheath enclosing the filament to protect it against the effect of animal fluids (Renon). Bayne-Jones did not believe that the club seen with *Actinomyces* was exclusively the result of interaction between the organism and the animal fluids (theories of Ravaut and Pinoy and of Magrou). He held that club formation could be produced in simple mediums free from serum and other animal protein. This was in contrast to the work of Wright,⁷⁷ who obtained granules (showing radiate formation) with *A. israeli* on broth to which had been added organic liquids, including blood, serum and pleural fluid. In 1 per cent dextrose-meat infusion agar and 1 per cent dextrose-meat infusion broth the filaments at the edges of the colonies were enclosed in sheaths of hyaline material, which terminated in bulbous thickenings over the ends of the filaments. The bulbous portion took no part in the growth as seen in hanging drop preparations. Growth of the filament took place away from the bulbous end, which apparently did not change. Similarly, Langeron, Cauchemez and Alleaux⁷⁸ were able to produce radiate forms of *Actinobacillus* on Sabouraud's dextrose agar without organic products, confirming Bayne-Jones' work, and in disagreement with the results of Ravaut and Pinoy, who obtained granules of *Actinobacillus* by growing the organism on a medium containing peptone and dextrose to which was added beef serum.

A different view regarding radiate formation was initiated by Ahlfeldt when she noticed that the prickles were found only on the adult organism of *C. immitis* when it was ready to liberate the young forms. This idea was taken up by Weidman, at least as regards *C. immitis*, who pointed out, in view of Ahlfeldt's observation, that radiation may be an accompaniment of reproductive processes when the reproductive structure is fully developed. It should be pointed out that this is not a rule and that many more mature cells have been

76 Bayne-Jones, S. J. Bact. **10** 569, 1925

77 Wright, J. H. J. M. Research **13** 349, 1905

78 Langeron, M., Cauchemez, L., and Alleaux, V. Ann. de parasitol. **3** 225, 1925

observed without the prickles or radiate formation than have been seen with them. Especially is this true of the fungus seen in the skin. It seems unlikely that the radiation effect is part of the process.

Nicaud⁷⁹ in 1928, while discussing the actinomycetoid form of *A. fumigatus*, revived some of the older theories when he concluded that "some organisms obtain an actinomycetoid form simply because of the associated growth of certain bacteria." Secondly, he pointed out that "in the case of *Aspergillus* radiations, this is a result of the influence of the medium, the reaction to the cells of the humors which perhaps modify by a digestive action the peripheral elements of the parasite, but it concerns elements which have conserved their vitality." This seems to fit in with the theories of Pinoy and Ravaut and Pinoy.

In 1932 Weidman¹⁹ analyzed much of the work already published and presented a discussion of radiate formation on a probable *Aspergillus* occurring in an infected capybara. In trying to ascertain whether the radiate formation was a product of the fungus or a contribution from the inflammatory processes involved, Weidman made careful studies of stained preparations from the animal. "In any event, the hyaloid material comprising the ray extended into direct contact with the cell in the interior of the fungus. Indeed, conditions were sometimes so favorable that the substance could be observed becoming integral with the fungicellulose in the wall of the micro-organism itself. I feel very strongly that this material is fungus in production and not host tissue." Haidenhain's iron-hematoxylin stain was also used by Weidman. This staining method colored the hyaloid substance black, and the wall of the cell took the red counterstain. "Again, the hyaloid incrustation was found to come into most intimate contact with the wall of the cell. However, the two did not blend, at least, in some cases the wall of the cell was recognizable as a pink, double-contoured shell independent of the rays." This appears to be a contradiction of the earlier statement. Later in the paper the author inclines to the view, "that the incrustation represents suppressed formations of the order of the Hülle cells (p. 739)." It is tempting, indeed, to assume that products of the micro-organism have diffused outward and coagulated or otherwise hyalinized surrounding fluids."

The incrustations of *Aspergillus* as described under the heading "Aspergillosis" do not appear to be "of the order of Hülle cells," as mentioned by Weidman. In the first place, these structures may occur anywhere on the filaments or hyphae and need not be limited to the terminal cells of the fungus, as is characteristic of Hülle cells. Secondly,

⁷⁹ Nicaud, P. *Compt. rend. Soc. de biol.* 99 1565, 1928.

they may occur as isolated nodules on the hypha, as interrupted nodules, or they may extend for some distance as a continuous sheath. They vary in size and shape. In fact, they are strongly reminiscent of the nodules of piedra on the hair. From their appearance alone they suggest incrustations.

Meyer⁸⁰ in 1934 concluded that the radiate formation on *Actinomyces* is not a form of degeneration or a defense mechanism set up by the organism, but a product of the host-parasite reaction. He favors Weidman's hypothesis that the radiate formation is produced by materials emanating from the organism. The bluish or pale violet tint observed in the granules he interprets as a lipid coming from the mycelium.

One of the most recent theories regarding radiate formation is that promulgated by Henrici⁸¹ in 1940. Henrici was attempting to obtain data on the mechanism of infection in deep-seated mycoses by producing aspergillosis experimentally in rabbits. He found that death could occur in these animals at two definite periods of time. In one period, within two days, death may follow inoculation with large doses of organisms and is attributed to an endotoxin produced by the fungus. In the second period, ten days or longer after inoculation, death results when the lesions change from abscesses to tubercles—a time when the animals have become hypersensitive to products of the fungus. He concluded from his observations "that after the infection has persisted for seven days or more, something happens which leads simultaneously to hypersensitivity, to an altered response of the host tissues, the tubercle, to an altered morphology of the fungus, the actinomycetoid form, and to a dissemination of the disease to new areas." He continues, "Although not yet proved, it seems a justifiable assumption that in experimental aspergillosis the actinomycetoid form of the fungus and the tuberculoid character of the lesion result somehow from the allergic state which develops." This is a new approach to the possible explanation of radiate formation. It is quite likely that the mechanism, whatever it may be, and the nature of the radiate material are essentially the same for all the diseases described in this paper.

In the absence of positive proof it is of course difficult to accept fully any one theory. One of the ideas set forth by the various authors may prove eventually to be the correct one.

Since the nature and the source of the radiating material are still in a theoretic or speculative stage, it is a temptation to add another possibility to the already long list. The work of Menkin⁸¹ on the dynamics of inflammation has brought to light a substance described as

80 Meyer, K. *Compt rend Soc de biol* **115** 1684, 1934

81 Menkin, V. *Dynamics of Inflammation. An Inquiry into the Mechanism of Infectious Processes*, New York, The Macmillan Company, 1940, p. 37

leukotaxine which can be isolated from inflammatory exudates. This substance has the property of attracting leukocytes (leukotactic), according to Menkin. When leukotaxine and the acidophilic substance are compared, there are many features of both which suggest a relationship. Both are crystalline material (doubly refractile) of a yellowish to brownish color. Both are insoluble in hydrochloric acid. According to Menkin, leukotaxine is soluble in glacial acetic and nitric acids. According to Israel, the radiating substance of the actinomycotic granule is insoluble in sulfuric and acetic acids. Both substances are insoluble in ether, chloroform or absolute alcohol. Heat does not effect either. Hydrolyzing of leukotaxine for about twenty hours in 9.5 normal sodium hydroxide inactivated it, whereas soaking of the granules of actinomycosis in alkali robbed the granules of their gloss and made them paler. Soaking of granules of aspergillosis for several days in 10 per cent potassium hydroxide changed the radiating substance, according to Weidman, to a finely granular matrix.

In view of the similar characteristics described it would seem logical to assume that the acidophilic substance is similar to or a form of leukotaxine. From a purely observational standpoint there are several points worthy of note. Chief among these are the facts that both substances are present in an inflammatory response and that leukocytes are found in abundance where both are concerned. Leukotaxine is a diffusible substance, and the radiate material tends to become a solid incrustation. This may be explained on the basis that the leukotaxine may become hardened as a result of its interaction with the fungous cell. On the other hand, diffusible material may crystallize about and on a foreign body, as is apparent in the initiation of crystal formation in mother liquor. Fungi, as has been pointed out in another publication,⁸² may be considered as actively proliferating foreign bodies.

In line with this approach to an understanding of the acidophilic substance, Berger, Vallee and Vezina⁸² favored the hypothesis that the radiating material was derived from the inflamed tissue, stating that "the clubs are not a direct result or product of the pathogenic agent, but seem to arise through a peculiar interaction between the agent and the surrounding exudative elements." In support of their belief they cited the work of Levaditi and Dimancesco-Nicolau,⁸³ who in 1926 obtained radiate formation in an experimental animal by injecting an oily suspension of tellurium intramuscularly. Sections of the muscle, ninety-nine days later, showed granulomas with masses of

82 Berger, L., Vallee, A., and Vezina, C. *Arch. Path.* **21**: 273, 1936.

83 Levaditi, C., and Dimancesco-Nicolau, O. *Compt. rend. Soc. de biol.* **95**: 531, 1926.

the injected inorganic substance surrounded by club formation. The radiate material appeared similar to that seen on actinomycotic granules and had the same tinctorial qualities. This work, of course, favors the theory that the radiations are produced by the host.

It seems justifiable, therefore, to study further the possible relationship of radiate formation and leukotaxine.

SUMMARY AND CONCLUSIONS

The phenomenon of radiate formation usually associated with actinomycosis, and consequently termed the actinomycetoid form of radiation, has been observed on fungi in human tissue. It is commonly encountered in actinomycosis, frequently observed in coccidioidal granuloma and occasionally noted in aspergillosis, it has been observed also in sporotrichosis, maduromycosis, paracoccidioidal granuloma, chromomycosis and blastomycosis. Pathologic study of the mycoses with which the radiation effect is associated reveals that in all cases there is a granulomatous response of the tissue accompanied by a variable degree of suppuration which is made manifest in some diseases, notably in actinomycosis and maduromycosis and to a lesser degree in coccidioidal granuloma and aspergillosis, by the formation of sinuses. In all instances there is a marked inflammatory reaction of the tissue, with massing of leukocytes in close association with the radiate form.

The radiating structures vary somewhat in shape and size. They may be of the actinomycetoid type, i.e., in the form of clubs or rays with broadened tips, they may assume the form of pickles or crystalline needles, tubular in appearance, they may appear as somewhat flattened hyaloid extensions, doubly refractile, with "broken-off" tips and lateral angular projections or they may be seen as peripheral nondescript, short extensions either partly or wholly surrounding the fungus. The unstained material is hyaloid, crystalline or ground-glass-like in appearance and yellow to yellowish green in color. These rays stain pink with eosin and consequently have been referred to as acidophilic substance.

Radiate formation is not a specific characteristic of the genus or of the species but may be found associated, with a varying degree of frequency, with any mycosis which produces an inflammatory reaction in the tissue that tends to persist and consequently becomes chronic and results in a granulomatous response.

The nature and the source of the radiating substance have not been definitely established. Several theories have been advanced in this regard, and these briefly are as follows: 1. The radiating mate-

rial is an aborted product of the fungus 2 It is living protoplasm capable of multiplying 3 It is the result of a host-parasite relationship—also a result of the digestive action of the host on the membrane of the fungus 4 It is a protective mechanism set up by the fungus (a diffusion product) 5 It is an accompaniment of a reproductive process 6 It is the result of an associated growth of certain bacteria 7 It results from the allergic state established by the organism in the tissue To these is added another possibility, namely, that the radiate substance may be similar or related to leukotaxine, described by Menkin

In addition to radiate formation, other phenomena have been noted on fungi in human tissue They consist of capsule formation as noted particularly on cells of *Cryptococcus* and *Histoplasma* and gloea formation, such as is found in hair infections (*pedia* and *lepothrix*) Also, cell wall projections can be seen on various spores both in tissue and in culture

THE LEUKOPENIC FACTOR OF EXUDATES

The Mechanism Concerned in the Leukopenia Induced by It

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PHILADELPHIA

IN A recent communication I demonstrated that exudates contain a leukopenic factor, particularly if at a p_H indicating acidity. On injection of such exudates sharp leukopenia ensues. This is subsequently followed by leukocytosis. The latter is to some extent referable to a leukocytosis-promoting factor (abbreviated as LPF) present in exudative material¹. The leukopenia occurs rapidly and lasts only several hours². The fact that such a factor is liberated at the site of an acute inflammation may be of significance in explaining numerous leukopenic states accompanying some well known inflammatory processes.

The question arises as to the mechanism of the leukopenia which develops after the injection of the leukopenic factor of exudates. The factor is often, though not exclusively, found to be in close association with pyrexin, the pyrogenic factor which is present in exudates and which per se offers a reasonable explanation of the mechanism of the fever produced with inflammation³. The present communication endeavors to throw further light on the possible mechanism whereby the leukopenic factor of exudates induces sharp transitory leukopenia.

EXPERIMENTS

After a basal white cell count had been made on blood of a dog (the blood obtained by nicking a superficial vessel of the ear lobe) whole exudate, the leukopenic factor or pyrexin was injected into the heart of the animal. The material was usually introduced either in the fluid state or after having been suspended in varying concentration in isotonic solution of sodium chloride. Within about a half hour the number of circulating leukocytes was found to have dropped impressively. After a short interval, when the number of white cells in the circulating blood was still low, i. e., when there was distinct leukopenia, the animal was killed. A careful postmortem examination was made. Representative samples of tissue from various organs were fixed in a 10 per cent solution of

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This communication represents paper no. 35 of a series entitled "Studies on Inflammation."

1 Menkin, V. (a) *Am J Path* **16** 13, 1940, (b) *Arch Path* **30** 363, 1940

2 Menkin, V. *Arch Path* **41** 50, 1946

3 Menkin, V. *Arch Path* **34** 28, 1945

formaldehyde or in Zenker's solution in which solution of formaldehyde U S P had been substituted for glacial acetic acid in the concentration of 5 per cent (Helly's modification) Some of the tissues were also fixed for staining of glycogen and fat As controls, some animals were studied after an intravascular injection of either isotonic solution of sodium chloride or some inert material

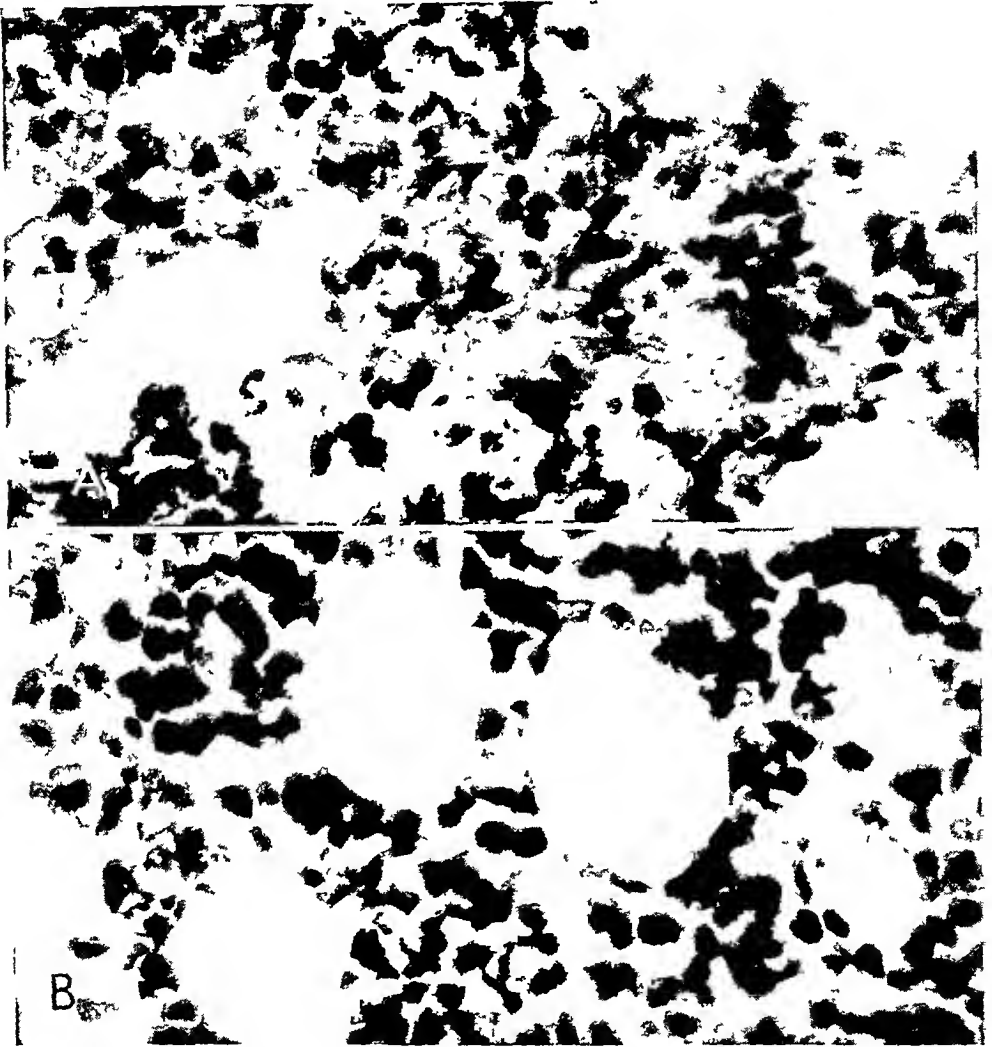


Fig 1—*A*, lung of dog 57-D The animal was given 26 mg of pyrexin by intracardiac injection and was killed during the leukopenic phase of the reaction Note the thickening of the alveolar wall due to the trapping of leukocytes ($\times 695$) *B*, bone marrow of the same dog The hyperplastic appearance of the marrow is probably largely referable to trapping of leukocytes owing to the rapidity of their occurrence following injection of the material ($\times 695$)

Microscopic sections revealed some interesting features At the height of the leukopenic state many leukocytes were found trapped in various parts of numerous organs, including the alveolar walls of the lungs, the sinusoids of the liver and the pulp of the spleen The bone marrow likewise apparently showed

retention of leukocytes⁴. Occasionally a few scattered leukocytes were found in the glomeruli of the kidney. Studies of differential blood smears indicated that the drop in the leukocyte count affected all types of leukocytes, i. e., the granulocytes as well as the monocytes. Some of the findings during the leukopenic phase are well illustrated by comparing a normal lung with that taken from an animal given an injection of the leukopenic factor (fig 1*A*). Figure 1*B*

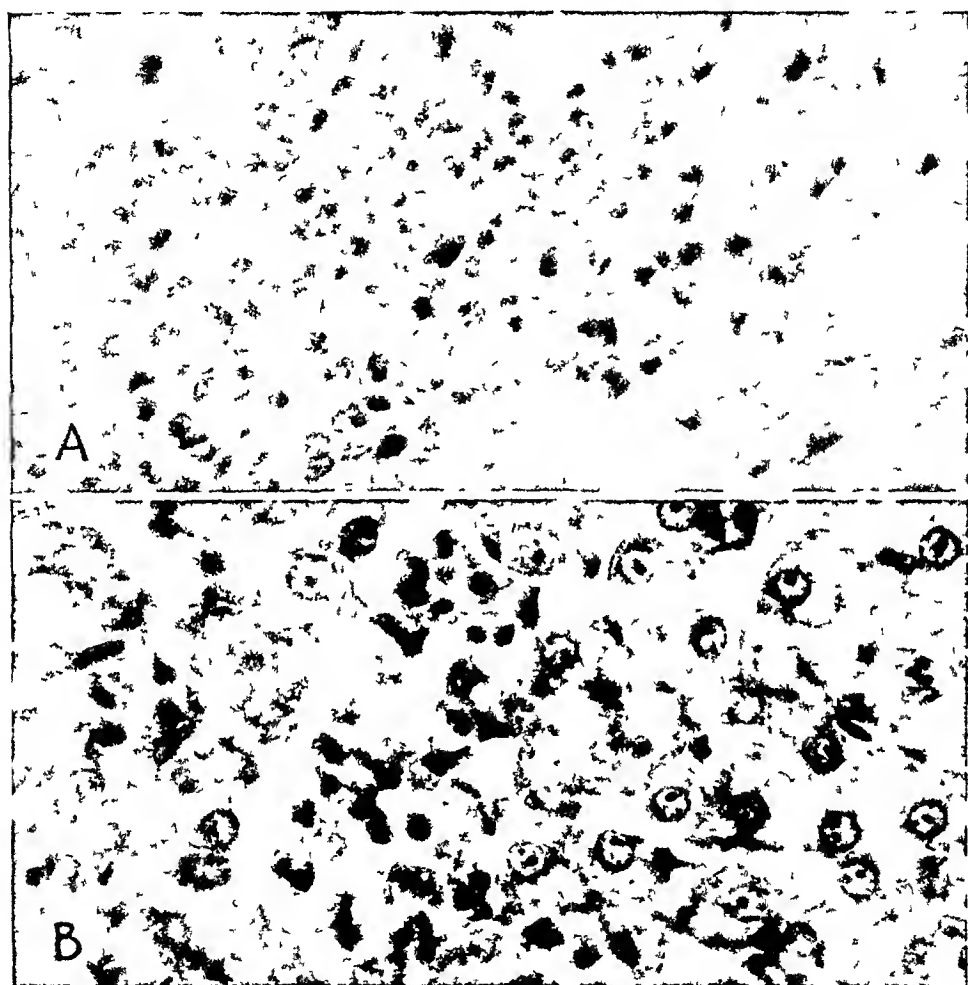


Fig 2—*A*, spleen of dog 57-D. This animal was killed during the leukopenic phase of the reaction following an injection of 26 mg of pyrexin. The multitude of islets of leukocytes in the spleen may explain in part the mechanism of the acute splenic tumor accompanying numerous inflammatory processes ($\times 695$). *B*, liver of the same dog. Leukocytes were caught in a number of sinusoids, as shown in the illustration ($\times 695$).

reveals the condition of the bone marrow during the leukopenic phase. In figure 2*A* is seen the trapping of leukocytes in the spleen. This splenic retention may perhaps be of help in elucidating further the mechanism of the acute splenic tumor accom-

4 It is conceivable in view of observations during the subsequent phase of leukocytosis that the picture of leukocytes being retained in the marrow is complicated by a superimposed hyperplastic response.

panying numerous inflammatory processes. The trapping of leukocytes in the sinusoids of the liver during the leukopenic phase is illustrated in figure 2B. It is quite possible that the leukocytosis which develops after a period of acute leukopenia is referable in part to release of leukocytes that had been trapped in various tissues. The observations, however, do not preclude compensatory hyperplasia of the bone marrow, which may increase the subsequent number of circulating leukocytes. This phase is being studied further. No trapping was found in the cutaneous vessels of 1 animal studied with this in mind.

Finally, it is of some interest to note that the liver and often the myocardial tissue revealed extensive deposits of glycogen. This is reminiscent of a similar state of affairs observed after repeated intravascular injections of necrosin⁵. This phase of the work is also being studied further. A summary of data from the various experiments appears in the table.

Summary of Experimental Data

| Dog | Dose of Pyrexin, Exudate or Leukopenic Factor Injected | Initial White Cell Count | White Cell Count Approx- imately at Time Animal Was Killed | Trapping of Leukocytes in Various Tissues | | | |
|------|---|--------------------------------|--|--|-------|--------|----------------|
| | | | | Lung | Liver | Spleen | Bone Marrow |
| 57 D | 26 mg pyrexin | 11,950 | 6,250 | + | + | + | + |
| 59-D | 23.5 mg pyrexin | 13,600 | 3,650 | + | + | + | + |
| 69 D | 6 cc purulent exudate (pH 5.2) | 10,750 | 3,350 | + | + | + | + |
| 73 D | 9 cc leukopenic factor | 12,050 | 4,400 | + (mild) | + | + | + |
| 77 D | 28 cc leukopenic factor | 15,600 | 5,750 | + | + | * | + |
| 80-D | 25 cc leukopenic factor | 16,350 | 4,200 | + | + | + | 0 |

+ This sign means that microscopic examination revealed trapping of leukocytes in the alveolar walls of the lung, in the sinusoids of the liver, in the splenic pulp (particularly around the malpighian corpuscles) and presumably in the marrow.

* Curiously enough, this animal showed an absence of the spleen.

COMMENT

The foregoing observations indicate that the leukopenia following the injection of an acid exudate, pyrexin or the leukopenic factor seems to be primarily referable to a trapping of leukocytes in the alveolar walls of the lung, in the sinusoids of the liver, in the pulp of the spleen and to a slight extent in the glomeruli of the kidney. There is apparently also trapping of leukocytes in the marrow, but studies made during the ensuing phase of leukocytosis suggest that besides trapping there may be compensatory hyperplasia in the marrow, probably to offset the abrupt leukopenic stage.

The findings are suggestive that the leukopenic factor of exudative material may be of significance in explaining the leukopenia which accompanies numerous inflammatory processes. It may be that the ultimate number of circulating leukocytes with inflammation depends in part on the resultant between two opposing factors in exudates. This is the leukopenic factor, on the one hand, which tends to depress the number of white cells, and the leukocytosis-promoting factor, on the other, which tends, in turn, to induce leukocytosis in the circulation.

The interplay of these two factors ultimately determines the actual number of circulating leukocytes

Finally it should be pointed out that the intravascular injections of necrosin,^{1b} of pyrexin and of the leukopenic factor are frequently accompanied by depositions of large amounts of glycogen in the hepatic cells and to some extent in cardiac muscle fibers. Whether in all these three fractions of exudates there is a common glycogen-producing factor remains to be determined. These studies are being pursued further.

SUMMARY

The leukopenia following the intravascular injection of an acid exudate, of pyrexin or of the leukopenic factor is accompanied by a trapping of apparently all types of leukocytes in the alveolar walls of the lungs, in the sinusoids of the liver, in the splenic pulp and perhaps in the marrow.

This trapping of leukocytes offers a reasonable explanation for the mechanism of the leukopenia following the injection of the leukopenic factor.

The trapping of leukocytes in the pulp of the spleen may serve to explain in a reasonable way the primary mechanism of the acute splenic tumor accompanying numerous inflammatory processes.

Pyrexin, the leukopenic factor and necrosin seem to contain a common glycogen-inducing factor such that injection of any of these substances is followed by deposition of large quantities of glycogen in the hepatic cells and to some extent in the cardiac muscle fibers. This factor is being investigated further.

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EXPERIMENTAL ENDOCARDITIS OF DOGS

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IT IS commonly thought that bacterial endocarditis does not occur unless the cardiac valves have been previously damaged. Thus Kinsella¹ stated that "two factors are necessary in every case, a preexisting injury of the valve, and a recent infection which may invade the blood stream." Willius² in a recent review commented that "an injured heart valve is a prerequisite for development of subacute bacterial endocarditis." Christian,³ however, stated that in only about 90 per cent of patients with bacterial endocarditis is there reason to believe that a previous injury of the heart valve existed. Christian's figures are more in accord with our own experience.

The earliest instance of experimental endocarditis produced without previous trauma of the cardiac valves was reported by Dreschfeld⁴ in 1887. Since that time numerous investigators, using the technic of intravenous injection of organisms, have reported widely varying results. This disagreement persists in even the more recent literature.

Rosenow⁵ produced endocarditis in normal rabbits by intravenous injections of streptococci isolated from the blood of patients with endocarditis.

MacNeal, Spence and Wasseen,⁶ using rabbits and a strain of *Streptococcus viridans* isolated from a patient with subacute bacterial endocarditis, noted that typical vegetative lesions developed in 27 of 57 animals. Kinsella and Muether⁷ were unable to produce endocarditis in dogs by injecting streptococci, however, after the valves had been

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1 Kinsella, R. A., in Cecil, R. L. *A Textbook of Medicine*, ed 6, Philadelphia, W. B. Saunders Company, 1944, p. 1074.

2 Willius, F. A. *Proc. Staff Meet., Mayo Clin.* **19**: 431, 1944.

3 Christian, H. A., in Osler, W. *Principles and Practice of Medicine*, ed 15, edited by H. A. Christian, New York, D. Appleton-Century Company, Inc., 1944, p. 1036.

4 Dreschfeld. *Brit. M. J.* **2**: 887, 1887.

5 Rosenow, E. C. *J. Infect. Dis.* **11**: 210, 1912.

6 MacNeal, W. J., Spence, M. J., and Wasseen, M. *Am. J. Path.* **15**: 695, 1939.

7 Kinsella, R. A., and Muether, R. O. *Arch. Int. Med.* **62**: 247, 1938.

injured mechanically, endocarditis could be produced by intravenous and even oral administration of the organisms

Loewe, Rosenblatt and Lederer,⁸ using five strains of *Str viridans*, induced endocarditis in 35 per cent of a series of rabbits. The incidence was increased by the selection of potent strains, whose virulence was enhanced by growth on enriched mediums and passage through mice.

Blahd, Frank and Saphir⁹ observed bacterial endocarditis in 40 per cent of 25 dogs whose heart valves were not previously injured, following one or more injections of streptococci isolated from the lung of a dog dying of pneumonia. They described the organisms as beta hemolytic streptococci. The authors suggested that the reason for their success, in contrast to the many failures previously reported in dogs, was that the strain of organisms employed was isolated from another dog, whereas other investigators had used bacteria isolated from human sources. Since these organisms were considered "substandard", their virulence was intensified by passing them through dogs not included in the series. Recently Loewe, Plummer, Niven and Sherman¹⁰ have attempted to show that only a particular type of *Str viridans* produces bacterial endocarditis.

METHOD OF STUDY

It has been our purpose to study this problem further and particularly to evaluate the importance of the type of organism, its virulence and the dosage in determining the incidence of endocarditis in dogs. A total of 99 animals were used, divided into two groups. In one group of 26 a planned effort was made to induce endocarditis. The other group, 73 animals, was given doses carefully regulated in an attempt to prevent the occurrence of endocarditis. In general, injections were given four times a week intravenously. The organisms were cul-

TABLE 1—*Incidence of Endocarditis Following Intravenous Injections of Bacteria*

| | Dogs | Number with Endocarditis | Percentage with Endocarditis |
|---------|------|-----------------------------|---------------------------------|
| Group 1 | 26 | 16 | 61.5 |
| Group 2 | 73 | 10 | 13.7 |
| Total | 99 | 26 | 26.2 |

tured in a broth of 0.3 per cent meat extract, 0.5 per cent salt and 1.0 per cent peptone with 0.2 cc sterile dog blood added to 150 cc of broth. Cultures were grown for twenty-four, forty-eight or seventy-two hours, depending on the rate of growth. No attempt was made to single out virulent organisms by testing of animals or to increase their virulence by special cultural methods. All organisms were isolated from routine clinical cultures, that is, from the noses, the throats,

8 Loewe, L., Rosenblatt, P., and Lederer, M. *Am J Path* **20** 89, 1944

9 Blahd, M., Frank, I., and Saphir, O. *Arch Path* **27** 424, 1939

10 Loewe, L., Plummer, N., Niven, C. F., and Sherman, J. M. *J A M A* **130** 257, 1946

the urine and the blood of normal patients and of patients with hypertension, scarlet fever, erysipelas and other diseases

In group 1 strains of *Str viridans* and of beta hemolytic streptococcus from thirteen different sources were used. In group 2 hemolytic and green-forming streptococci, diphtheria bacilli, staphylococci, colon bacilli, pneumococci and *Bacillus mucosus* were employed in addition to a number of those used in group 1. The organisms were obtained from forty-six sources.

Nineteen animals of group 1 were initially given large doses, varying from 50 to 100 cc of broth, the remaining dogs received small doses, from 10 cc to 50 cc. Fifty-one dogs of group 2 were initially given small doses, and 22 received large doses.

TABLE 2—*Data on Dogs with Endocarditis*

| Dog | Organism | Source | Time of Death | Location |
|-----|------------------------------|---------------------------------|---------------|--------------------------|
| 1 | Beta hemolytic streptococcus | Erysipelas | 21 months | Aortic valve |
| 2 | Beta hemolytic streptococcus | Erysipelas | 11 months | Mitral valve |
| 3 | Beta hemolytic streptococcus | Scarlet fever | 16 months | Mitral valve |
| 4 | Beta hemolytic streptococcus | Pneumonia | 2 months | Aortic valve |
| 5 | Beta hemolytic streptococcus | Pneumonia | 6 days | Mitral valve |
| 6 | Beta hemolytic streptococcus | Pneumonia | 6 days | Aortic and mitral valves |
| 7 | Beta hemolytic streptococcus | Pneumonia | 25 days | Mitral valve |
| 8 | Beta hemolytic streptococcus | Pneumonia | 11 days | Mitral valve |
| 9 | Beta hemolytic streptococcus | Pneumonia | 17 days | Mitral valve |
| 10 | Beta hemolytic streptococcus | Pneumonia | 7 months | Mitral and aortic valves |
| 11 | Beta hemolytic streptococcus | Scarlet fever | 22 months | Mitral and aortic valves |
| 12 | <i>Str viridans</i> | Culture of material from nose | 35 months | Mitral and aortic valves |
| 13 | <i>Str viridans</i> | Subacute bacterial endocarditis | 5 months | Aortic valve |
| 14 | <i>Str viridans</i> | Subacute bacterial endocarditis | 7+ months | Aortic valve |
| 15 | <i>Str viridans</i> | Culture of material from nose | 12 days | Mitral valve |
| 16 | <i>Str viridans</i> | Blood | 1 month | Mitral valve |
| 17 | <i>Str viridans</i> | Urine | 2 months | Mitral valve |
| 18 | <i>Str viridans</i> | Urine | 12 months | Mitral valve |
| 19 | <i>Str viridans</i> | Urine | 27½ months | Mitral valve |
| 20 | <i>Str viridans</i> | Urine | 2 months | Mitral valve |
| 21 | Beta hemolytic streptococcus | Throat | 33 months | Mitral valve |
| 22 | Beta hemolytic streptococcus | Pneumonia | 82½ months | Mitral valve |
| 23 | Beta hemolytic streptococcus | Tonsil | 15½ months | Mitral valve |
| 24 | <i>Str viridans</i> | Tooth | 5 months | Mitral valve |
| 25 | Beta hemolytic streptococcus | Throat | 71½ months | Mitral valve |
| 26 | <i>Str viridans</i> | Urine | 24 months | Mitral valve |

Group 2 was made up of animals which were used to produce experimental hypertension. This work has recently been reported.¹¹ In this series, the frequency with which endocarditis was obtained in the first series was kept in mind, and an effort was made to avoid endocarditis by keeping the doses low and discontinuing the injections for a time when untoward effects appeared, such as loss of weight, anorexia and fever.

RESULTS

Bacterial endocarditis developed in 26 of the 99 dogs. The organisms used were of 44 strains. Of these, 19 caused endocarditis in 1 or more instances. These figures are far more revealing, however, when broken down into two groups. In group 1 16 dogs showed autopsy-proved bacterial endocarditis. In 10 of the 73 animals of group 2 endo-

¹¹ Dick, G. F. Arch Path 39 81, 1945

carditis developed despite every effort made to prevent such an occurrence

No strains other than those of *Str viridans* and beta hemolytic streptococcus induced endocarditis (These two organisms constituted more than 84 per cent of the strains used) In 15 cases, beta hemolytic streptococcus was responsible, and in 11 *Str viridans* Since 46 animals were given the beta hemolytic and 37 the viridans type, it appears that the respective incidence is approximately the same That this is a real and not an apparent distribution is borne out by the fact that the dosages of the organisms used in groups 1 and 2 were essentially the same

TABLE 3—*Incidence of Causative Organisms in Cases of Endocarditis*

| | Dogs Inoculated | Number with Endocarditis | Percentage with Endocarditis |
|---|--------------------|-----------------------------|---------------------------------|
| Beta hemolytic streptococcus | 46 | 15 | 32 |
| <i>Str viridans</i> | 37 | 11 | 30 |
| Other organisms—diphtheria bacilli, <i>B mucosus</i> , etc | 16 | 0 | 0 |

SUMMARY

A total of 99 dogs were given repeated intravenous injections of a variety of organisms isolated in routine cultures of all types Forty-four different strains, chiefly strains of *Str viridans* and beta hemolytic streptococcus, were used No attempt was made to select strains or to increase their virulence The dogs were placed in two groups group 1, in which a definite attempt was made to induce endocarditis, and group 2, in which efforts were directed toward preventing its occurrence In 61 per cent of group 1 endocarditis developed and, despite all precautions, 13 per cent of group 2 showed lesions of the same type Positive results were obtained with the same frequency whether *Str viridans* or beta hemolytic streptococcus was used It has been our experience that the difficulty lies more in preventing occurrence of endocarditis than in producing it if streptococci are injected intravenously into dogs for any considerable period

CONCLUSIONS

Contrary to existing opinion, bacterial endocarditis may be produced in dogs without previous injury of the cardiac valves

It is not necessary to use streptococci from sites of endocarditis, as streptococci from a great variety of sources produce the disease in dogs

While virulent strains produce endocarditis in shorter time and with fewer injections than do the less virulent strains, it is necessary only to continue the injections of the less virulent ones for a longer time and in increased doses to produce endocarditis regularly

IN VITRO STUDIES ON THE PHYSIOLOGY OF CELLS

Interactions of Thymic Cells and an Oxidation-Reduction Indicator,
2,6-Dichlorophenolindophenol

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THE PHYSIOLOGIC reactions of cells have been studied in this laboratory by (1) the method of unstained cell counts, (2) the deferred histologic method and (3) the electrometric determination of the hydrogen ion concentrations of cellular suspensions¹ These three methods are based on three distinct physiologic capacities of cells, namely (1) the capacity of viable cells to resist staining with eosin; (2) the capacity of excised viable tissues to react histologically to reagents and (3) the capacity of cells to ferment glucose, with formation of acid The methods have been found useful in studies on the reactions of cells to physical, chemical and biologic reagents such as oxygen, glucose, antiseptics, distilled water and roentgen rays

A fourth physiologic capacity appears in the oxidation-reduction activity of cells The present investigation is a preliminary study to determine whether this function can be used to develop another method of measuring the reactions of cells to reagents in vitro

Oxidation-reduction activities have been studied extensively in suspensions of bacteria by Quastel,² Coulter and Isaacs,³ Clifton⁴ and Burrows and Jordan⁵ The oxidation-reduction properties of suspended animal cells have been investigated by Drew,⁶ Voegtlin, Johnson and Dyer,⁷ Cannan, Cohen and Clark⁸ and Chambers, Beck and Green⁹

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1 Schrek, R (a) *Proc Soc Exper Biol & Med* **54** 283, 1943, (b) *Arch Path* **35** 857, 1943, (c) **37** 319, 1944, (d) *Am J Path* **21** 1101, 1945, (e) *Radiology* **46** 395, 1946

2 Quastel, J H *Biochem J* **20** 166, 1926

3 Coulter, C B, and Isaacs, M L *J Exper Med* **49** 711, 1929

4 Clifton, C E *J Bact* **25** 495, 1933

5 Burrows, W, and Jordan, E O *J Infect Dis* **58** 259, 1936

6 Drew, A H *Brit J Exper Path* **1** 115, 1920

7 Voegtlin, C, Johnson, J M, and Dyer, H A *J Pharmacol & Exper. Therap* **24** 305, 1924

(Footnotes continued on next page)

The present communication reports observations on the reactions of thymic cells in suspension to an oxidation-reduction indicator, 2,6-dichlorophenolindophenol

REDUCTION OF 2,6-DICHLOROPHENOLINDOPHENOL BY THYMIC CELLS

Effect of Mediums—A suspension of thymic cells in phosphate-Ringer solution was prepared by the methods outline in a previous paper^{1c} To 0.2 cc of this suspension was added 0.1 cc of the reagent studied (such as serum or a solution of glucose or a phosphate-Ringer solution) and 0.2 cc of 2,6-dichlorophenolindophenol dissolved in phosphate-Ringer solution. The controls consisted of mixtures of comparable amounts of reagent and indicator without the addition of thymic cells. The test tubes were shaken horizontally one hundred and forty times per minute in a water bath maintained at 37°C. The mixtures were examined at intervals to determine the intensity of color. It was difficult to compare the experimental mixtures containing thymic cells and the control solutions without cells. Suspensions showing the indicator completely or partially

TABLE 1—*Color Intensity of 2,6-Dichlorophenolindophenol Immediately and One Hour After Its Addition to a Mixture of Rabbit Thymic Cells and Reagent*

| Final Concentration of Indicator | Intensity of Color with Reagent Added * | | | | |
|----------------------------------|---|--------------|--------------------------|--------------------------|---------------------------|
| | Phosphate Ringer Solution | Rabbit Serum | Glucose ^a M/6 | Mannose ^a M/6 | Fructose ^a M/6 |
| 1 5,000 | 5 5 | 4 2† | 5 - 5 | 5 5 | 5 5 |
| 1 10,000 | 4 4 | 3 1† | 4 - 4 | 4 4 | 4 4 |
| 1 20,000 | 3 3 | 2 - 0† | 2 2 | 2 2 | 3 3 |
| 1 40,000 | 0 2 | 2 0† | 0 0† | 0 0† | 0 2 |
| 1 80,000 | 0 1 | 1 0† | 0 0† | 0 0† | 0 0† |
| 1 160,000 | 0 0† | 1 - 0† | 0 0† | 0 0† | 0 0† |
| None | 0 0 | 0 - 0 | 0 0 | 0 0 | 0 0 |

* The numbers indicate the intensity of the color of the indicator in the suspension. 5 representing maximal 1, minimal 0, no bluish color. The first number represents the intensity in color immediately after the addition of the indicator, the second number after one hour of incubation at 37°C.

† The bluish color of the indicator was restored or intensified on the addition of potassium ferricyanide in a 0.1 molar solution.

decolorized in one hour were tested by the addition of 0.1 cc of potassium ferricyanide (0.1 molar) to see whether the reduced dye could be reoxidized and the blue color restored.

A summary of several experiments on the reduction of the indicator by thymic cells is presented in table 1. It was observed that immediately after the dye in dilutions of 1 40,000 and 1 80,000 was added to the cells in a phosphate-Ringer solution the blue color of the indicator disappeared (table 1) but that it returned after a few minutes of incubation. The indicator in a dilution of 1 160,000 was immediately reduced by the thymic cells and remained reduced during the one hour incubation period. It seems, then, that washed thymic cells in phosphate-Ringer solution had the capacity of reducing 2,6-dichlorophenolindophenol under aerobic conditions only when the solution of the dye was very dilute (1 160,000).

8 Cannan, R. K., Cohen, B., and Clark, W. M., in *Studies on Oxidation-Reduction*, Hygienic Laboratory Bulletin no. 151, United States Public Health Service, 1928, p. 306.

9 Chambers, R., Beck, L. V., and Green, D. E. *J. Exper. Biol.* 10:142, 1933.

Table 1 shows that in the presence of a small amount of homologous serum the thymic cells of the rabbit had decolorized completely a 1:20,000 solution of dye after one hour's incubation. Thymic cells of the rat in the presence of rat serum decolorized the dye to the same degree as the cells of the rabbit. It would seem, then, that thymic cells readily reduced 2,6-dichlorophenolindophenol even when the concentration was fairly high, in the presence of a small amount of homologous serum.

Mixtures of a suspension of thymic cells and a solution of glucose or mannose completely reduced the indicator in concentrations of 1:40,000 (table 1). The addition of fructose increased to a slight extent the capacity of thymic cells to reduce the indicator. In a previous work it had been shown that thymic cells can ferment glucose and mannose and possibly galactose but not other sugars.¹⁰ It would seem from these experiments that the capacity of thymic cells to reduce 2,6-dichlorophenolindophenol is increased by the addition of glucose, mannose or, to a lesser extent, fructose.

Effect of Solution of Formaldehyde U S P—Several experiments were performed to determine whether the degree to which thymic cells reduce the indicator in the presence of serum could be affected by the addition of a toxic reagent such as formaldehyde. Solution of formaldehyde U S P was diluted with phosphate-Ringer solution, and 0.1 cc of each dilution was mixed with 0.2 cc of thymic cell suspension, 0.1 cc of rabbit serum and 0.2 cc of a dilution of the indicator. The mixtures were incubated and examined for color periodically. The results of these experiments are summarized in table 2. It is seen from table 2 that when the concentration of solution of formaldehyde U S P was very low, 1:7,680, the reagent had no perceptible effect on the capacity of the cells to reduce the dye in the presence of serum. A greater concentration of solution of formaldehyde U S P, 1:1,920, permitted rapid reduction of the indicator, but after four hours of incubation the bluish color of the indicator was restored spontaneously. This concentration of solution of formaldehyde U S P had, then, a delayed effect on the capacity of the cells to reduce the indicator. With a still higher concentration of the reagent, 1:60, the decolorization of the indicator was inhibited. There is, then, an immediate and a delayed effect of formaldehyde on the cellular reduction of 2,6-dichlorophenolindophenol. With lower concentrations of the reagent, only the delayed response, namely reoxidation of the indicator, was observed. With higher concentrations of formaldehyde the immediate response, an inhibition of the reduction of the indicator, was also seen.

In one experiment the cellular suspension was diluted with an equal amount of phosphate-Ringer solution to reduce the number of cells. With the original, undiluted suspension, solution of formaldehyde U S P diluted 1:60 inhibited the reduction of the indicator (table 2). With the diluted cellular suspension a lower concentration of solution of formaldehyde U S P, 1:120, sufficed to inhibit the complete reduction of the indicator. Evidently, the amount of solution of formaldehyde U S P needed to inhibit the decolorization of the indicator varied directly with the number of cells in the suspension.

It is seen in table 2 that with a low concentration of the indicator, 1:40,000, there was required a fairly high concentration of solution of formaldehyde U S P, 1:60, to inhibit the reducing action of the thymic cells. With a higher concentration of the dye, 1:20,000, a lesser concentration of solution of formaldehyde U S P, 1:240, sufficed to inhibit the reduction of the indicator. The higher

the concentration of the dye, the lesser was the concentration of solution of formaldehyde U S P required to inhibit reduction. It may be concluded that the amount of solution of formaldehyde U S P required for inhibition of reduction varied directly with the number of cells and varied inversely with the concentration of the indicator.

One would assume that the observed inhibiting action of solution of formaldehyde U S P on the reduction of the indicator may be due to a cytoeidal action of the reagent on the thymic cells. This hypothesis was tested by the addition of eosin in Tyrode's solution in order to stain the dead cells. It was found, however, that all the cells were unstained or showed only a minimal amount of staining. Further experiments revealed that formaldehyde interfered with the eosin staining of cells known to be dead. The cytoeidal action of formaldehyde cannot be measured by the method of counting eosin-resistant cells.

TABLE 2—*Effect of Solution of Formaldehyde U S P on the Capacity of Rabbit Thymic Cells to Reduce 2,6-Dichlorophenolindophenol in the Presence of Rabbit Serum*

| Concentration of indicator | Intensity of Color of Indicator Incubated with Cells, Serum and Solution of Formaldehyde U S P * | | | | | | | | |
|--|--|----|-----|----------|----|-----|----|----|-----|
| | 1 20,000 | | | 1 40,000 | | | | | |
| | 70 | | | 70 | | | 15 | | |
| | 20 | 60 | 240 | 20 | 60 | 240 | 20 | 60 | 240 |
| Minutes of incubation | | | | | | | | | |
| Final concentration of solution of formaldehyde, U S P | | | | | | | | | |
| 1 60 | 3 | 3 | 3 | 1 | 2 | 2 | 2 | 2 | 2 |
| 1 120 | 3 | 3 | 3 | 0 | 0 | 2 | 1 | 1 | 2 |
| 1 240 | 2 | 3 | 3 | 0 | 0 | 2 | 0 | 0 | 2 |
| 1 480 | 0 | 3 | 3 | 0 | 0 | 2 | 0 | 0 | 2 |
| 1 960 | 0 | 3 | 3 | 0 | 0 | 2 | | | |
| 1 1,920 | 0 | 0 | 2 | 0 | 0 | 1 | | | |
| 1 3,840 | 0 | 0 | 1 | 0 | 0 | 0 | | | |
| 1 7,680 | 0 | 0 | 0 | 0 | 0 | 0 | | | |
| None | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* The numbers indicate the intensity of the color of the indicator, as explained in table 1

EFFECT OF 2,6-DICHLOROPHENOLINDOPHENOL ON GLYCOLYSIS

A suspension of rat thymic cells was incubated for one and four hours with a solution of glucose (M/30) and the oxidation-reduction indicator. The hydrogen ion concentrations of the mixtures were then determined by means of an electrometer equipped with a glass electrode and microchamber. Similar mixtures were incubated anaerobically in capillary glass tubes sealed with rubber tubing. The findings of one experiment are presented in table 3.

The p_H of a control thymic cell suspension incubated aerobically for one hour without the indicator was 7.35 in the absence of glucose and 6.98 in its presence. Evidently the amount of acid produced was only slight on aerobic incubation, i.e., aerobic glycolysis of thymic cells is low.

The effect of 2,6-dichlorophenolindophenol on aerobic glycolysis depended on the concentration of the dye. The p_H of the cell suspension-glucose solution mixture after one hour of incubation was decreased by moderate concentrations of the indicator (6.98 in the absence and 6.40 in the presence of a 1:40,000 concentration) but was not affected by low (1:160,000) or high (1:5,000) concen-

trations of the indicator Apparently aerobic glycolysis is stimulated by moderate amounts but is not affected by low or excessive amounts of the indicator

Under anaerobic conditions, in the absence of the indicator the p_H of the suspensions incubated for one hour was 7.08 in the absence and 6.03 in the presence of glucose Anaerobic glycolysis is, then, quite marked The anaerobic production of acid was slightly increased (p_H 5.84) by moderate concentrations of the indicator (1:40,000) and was markedly inhibited (p_H 6.95) by high concentrations (1:5,000)

It appears from these studies that glycolysis is high under anaerobic conditions or in the presence of a moderate concentration of the indicator Glycolysis is low under aerobic conditions or in the presence of a high concentration of the indicator

TOXICITY OF 2,6-DICHLOROPHENOLINDOPHENOL FOR THYMIC CELLS

Susceptibility of Rat and Rabbit Thymic Cells in Phosphate-Ringer Solution

—The toxicity of the dye for thymic cells of the rabbit was studied by the method of unstained cell counts^{1b} Mixtures of cells, reagent and dye were incubated

TABLE 3—*Effect of 2,6-Dichlorophenolindophenol on the Hydrogen Ion Concentration of Suspensions of Rat Thymic Cells Incubated with Glucose Under Aerobic and Anaerobic Conditions*

| Final Concentration of Indicator | p_H of Mixture | | |
|--|------------------------|-------------------------|--|
| | One Hour of Incubation | | Four Hours of Incubation Aerobic Conditions |
| | Aerobic Conditions | Anaerobic Conditions | |
| 1:5,000 | 7.14 (5)* | 6.95 (3) | 7.10 (5) |
| 1:10,000 | 6.82 (4) | 6.84 (1) | 6.84 (4) |
| 1:20,000 | 6.65 (3) | 6.49 (0) | 6.24 (3) |
| 1:40,000 | 6.40 (1) | 5.87 (0) | 5.62 (1) |
| 1:80,000 | 6.81 (0) | 5.84 (0) | 6.78 (0) |
| 1:160,000 | 6.90 (0) | 5.95 (0) | 6.96 (0) |
| 1:320,000 | 6.95 (0) | 5.97 (0) | 7.02 (0) |
| None | 6.98 (0) | 6.03 (0) | 7.06 (0) |

* The numbers in parentheses indicate the intensity of color of the indicator, as explained in table 1

at 37°C, and eosin, 1:1,000 in Tyrode's solution, was then added A drop of the resulting mixture was placed in a hemocytometer The unstained cells, the eosin-stained cells and the red blood cells were counted For reasons presented in a previous paper^{1b} the unstained cells were assumed to be viable and the stained cells were considered dead

Table 4 shows the effect of the indicator on the number of unstained thymic cells in various mediums In the first experiment to be considered, the thymic cells were obtained from a rabbit, and the medium consisted of phosphate-Ringer solution Incubation had no appreciable effect on the number of unstained thymic cells in phosphate-Ringer solution without indicator (92.8 cells per millimicroliter before and 94.8 cells after an incubation period of two hours) Addition of the dye in 1:160,000 dilution caused a significant decrease in the number of unstained cells (from 92.8 to 40.4) A higher concentration of dye, 1:40,000 caused almost complete disappearance of the cells resistant to eosin (only 0.2 unstained cell per millimicroliter left) In this and in many other experiments it was definite that 2,6-dichlorophenolindophenol was extremely cytotoxic to rabbit thymic cells in the presence of phosphate-Ringer solution

A similar experiment was conducted with thymic cells of the rat. A 1:10,000 concentration of the dye failed to cause any definite decrease in the number of unstained cells (624 unstained cells per millimicroliter before and 516 after incubation, table 6). A concentration of 1:5,000 caused only a moderate decrease to 252 unstained cells. This is in sharp contrast to the experiment with the cells of the rabbit, in which a dilution of 1:80,000 was extremely toxic and reduced the number of unstained cells from 928 to 70. The 2,6-dichlorophenol-indophenol was markedly cytotoxic to rabbit thymic cells in phosphate-Ringer solution but had only a minimal lethal action on the cells of the rat.

The question arises whether the toxic action of the indicator on rabbit thymic cells was due to light which had been absorbed and activated by the dye. To eliminate the action of light, the suspension and the dye were pipetted into test tubes which had been blackened on the outside by immersion in black enamel paint. The layer of paint was sufficiently heavy so that it was not possible to see the fluid in the test tubes even with bright illumination. The reagents were not protected from light before mixing. The mixtures of thymic cells and dye were

TABLE 4—*Toxicity of 2,6-Dichlorophenolindophenol for Thymic Cells in Various Mediums*

| Final Concentration of Dye | Number of Unstained Cells per Millimicroliter After Incubation of Dye, Thymic Cells and Reagent for Two Hours | | | | | |
|----------------------------|---|---------------------------|--------------|-------------|-------------|--------------|
| | Rat Thymic Cells | Rabbit Thymic Cells | | | | |
| | Phosphate Ringer Solution | Phosphate Ringer Solution | Rabbit Serum | Glucose M/G | Mannose M/G | Fructose M/G |
| 1:5,000 | 252 (5)* | 03 (5) | 40 (5) | | 03 (5) | 00 (5) |
| 1:10,000 | 516 (5) | 00 (4) | 672 (3) | 84 (3) | 22 (4) | 03 (4) |
| 1:20,000 | 520 (4) | 02 (3) | 976 (1) | 564 (0) | 632 (1) | 05 (3) |
| 1:40,000 | 592 (3) | 02 (2) | 964 (0) | 876 (0) | 768 (0) | 13 (2) |
| 1:80,000 | 536 (2) | 70 (1) | | 804 (0) | 976 (0) | 900 (0) |
| 1:160,000 | | 404 (0) | | | 884 (0) | 768 (0) |
| None | 524 | 948 | 940 | 970 | 944 | 976 |
| Before incubation | 624 | 928 | 928 | 1032 | 936 | 936 |

* The numbers in parentheses indicate the intensity of color of the indicator, as explained in table 1.

incubated in the blackened test tubes and in ordinary tubes. After two hours of incubation, eosin was added to the mixtures. A drop was placed in the hemocytometer, and the cells were immediately examined and counted. It was found that there was no appreciable difference in the unstained cell counts of the mixtures in the painted and in ordinary test tubes. Apparently, the toxic action of 2,6-dichlorophenolindophenol on thymic cells of the rabbit was not due to the dynamic action of light.

Rate of Toxic Reaction—The rate of the cytotoxic action of the dye was determined as follows. A solution of the indicator was sterilized by passing it through a Seitz filter. A sterile suspension of thymic cells of the rabbit was incubated with the indicator at 37°C. Unstained cell counts were made periodically. The data were represented graphically on logarithmic-probability paper, and the 50 and 10 per cent survival periods were estimated from the graphs. It was observed that 90 per cent of the cells died in 0.7 hour when incubated with indicator 1:20,000, in 3.1 hours with indicator 1:80,000, in 7.6 hours with indicator 1:160,000 and in 42.5 hours in the absence of indicator. It would seem that the toxic action of the higher concentrations of the indicator was rapid.

Even very low concentrations had a perceptible toxic action on thymic cells of the rabbit

Effect of Serum—The medium of the experiments on the toxic action of the indicator was a phosphate-Ringer solution. The effect of the addition of serum is shown in table 4. It was observed that the indicator even in a concentration of 1:20,000 failed to cause any appreciable change in the unstained cell count in the presence of serum (97.6 and 94.0 cells per millimicroliter with and without dye, respectively). Evidently, rabbit serum inhibited the cytotoxic action of 2,6-dichlorophenolindophenol on thymic cells of the rabbit.

Further experiments were done to determine how much serum was needed to neutralize the toxic effect of the dye. It is seen from table 5 that with a dye concentration of 1:40,000 it took 0.025 cc of serum to neutralize the toxic effect, with a concentration of 1:20,000, 0.05 cc of serum, and with a concentration of 1:10,000, 0.1 cc. In this roughly quantitative experiment it appeared that the greater the amount of dye used the greater was the amount of serum required to neutralize the toxicity. The volume of serum needed was approximately proportional to the concentration of the dye.

TABLE 5—*Effect of Serum on the Cytotoxic Action of 2,6-Dichlorophenolindophenol*

| Concentration of indicator Dilution of suspension of thymic cells of rabbit Cc of serum added | Number of Unstained Cells per Millimicroliter After Two Hours of Incubation | | | | |
|--|--|-----------|-----------|-------------|-------------|
| | 1:10,000 | 1:20,000 | 1:40,000 | 1:40,000 | 1:40,000 |
| | Undiluted | Undiluted | Undiluted | Diluted 1:2 | Diluted 1:4 |
| 0.1 | 82.4 (0)* | 69.2 (0) | 72.4 (0) | 37.6 (1) | 17.6 (2) |
| 0.05 | 1.8 (3) | 63.6 (1) | 78.0 (0) | 40.4 (1) | 14.0 (2) |
| 0.025 | 0.5 (4) | 0.3 (2) | 85.6 (0) | 32.0 (2) | 0.3 (3) |
| 0.0125 | 0.0 (4) | 0.0 (4) | 1.6 (2) | 0.9 (3) | 0.0 (3) |
| 0.0062 | 0.0 (4) | 0.0 (4) | 0.7 (2) | 1.4 (3) | 0.0 (4) |
| 0.0031 | 0.0 (4) | 0.2 (4) | 0.6 (2) | 0.2 (3) | 0.0 (4) |
| None | 0.3 (4) | 0.0 (4) | 0.6 (2) | 0.0 (3) | 1.4 (4) |

* The numbers in parentheses indicate the intensity of color of the indicator, as explained in table 1.

In another experiment summarized in table 5, the dye was kept constant, and the serum and the cells were the variable factors. The undiluted cellular suspension required approximately 0.025 cc of serum to protect the thymic cells against the toxic action of dye in a 1:40,000 dilution. The suspension diluted 1:4 necessitated only a slightly larger amount of serum, 0.05 cc, for protection against the same amount of dye. In this and in other experiments it appeared that when the concentration of the dye was kept constant a lesser number of cells required a slightly larger amount of serum for protection against the cytotoxic action of the dye. The amount of serum required then varied inversely with the number of cells in the suspension and was directly proportional to the concentration of the dye.

Effect of Glucose—In experiments similar to those just described, glucose was substituted for serum. According to table 4, this reagent protected thymic cells of the rabbit against the toxic action of the indicator in a dilution of 1:40,000 (87.6 and 90.0 cells per millimicroliter, with and without dye). The same concentration of dye caused an almost complete disappearance of eosin-resistant cells in the absence of glucose (0.2 and 94.8 unstained cells, with and without dye). It may be concluded, then, that glucose caused the reduction of the indicator and

protected the thymic cells of the rabbit against the cytotoxic action of 2,6-dichlorophenolindophenol

An experiment was performed to determine how much dextrose is required to neutralize the toxic effect of the dye. With a concentration of indicator of 1/40,000 and a M/7290 solution of glucose, the number of unstained cells dropped from 700 to 130 cells per millimicroliter (table 6). With the dye in a 1/20,000 dilution, a M/810 solution of glucose was required to protect 125 cells per millimicroliter. When the concentration of the indicator was 1/10,000, the maximum amount of glucose used (M/30) protected only 76 cells per millimicroliter. It would seem, then, that a moderate increase in the amount of indicator added necessitated a considerable increase in the glucose required for the protection of the cells. With the serum, on the other hand, a moderate increase in the dye required only a proportional increase in the amount of serum needed.

TABLE 6—*Effect of Glucose on the Cytotoxic Action of 2,6-Dichlorophenolindophenol*

| | Number of Unstained Cells per Millimicroliter After Two Hours of Incubation | | | | |
|--|--|----------|----------|----------|-----------|
| | 1/10,000 | 1/20,000 | 1/40,000 | 1/80,000 | 1/160,000 |
| Concentration of indicator | | | | | |
| Number of unstained thymic cells of rabbit before incubation | 700 | 700 | 700 | 236 | 152 |
| Concentration of glucose, molarity | | | | | |
| 1/30 | 76 (4)* | 46.4 (1) | 76.4 (0) | 24.8 (0) | 52 (1) |
| 1/90 | 64 (4) | 45.2 (1) | 64.8 (0) | 25.6 (0) | 28 (1) |
| 1/270 | 40 (4) | 28.8 (2) | 65.2 (0) | 20.8 (0) | 31 (1) |
| 1/810 | 26 (4) | 12.5 (3) | 55.6 (0) | 8.0 (1) | 31 (2) |
| 1/2,430 | 07 (4) | 04 (3) | 10.8 (1) | 2.7 (2) | |
| 1/7,290 | | 05 (3) | 13.0 (1) | | |
| 1/21,870 | | | 2.6 (2) | | |
| None | 06 (5) | 00 (3) | 1.2 (2) | 07 (2) | 08 (2) |

* The numbers in parentheses indicate the intensity of color of the indicator as explained in table 1.

The effect of varying the number of cells in suspension is also shown in table 6. When the mixtures contained the undiluted cellular suspension (70 cells per millimicroliter), a M/810 solution of dextrose was required for protection of the cells. With the suspension diluted approximately 1/4 (152 cells per millimicroliter), even a M/30 solution of glucose protected only one third of the cells. Evidently, then, the higher the dilution of cells, the greater was the amount of glucose required to protect the cells against the toxic effect of 2,6-dichlorophenolindophenol.

COMMENT

The objectives of the present and previous investigations on cellular physiology are (1) to develop methods for studying the physiology of cells and (2) to determine differences in the physiologic reactions of the various types of cells. In the present phase of this work the dye 2,6-dichlorophenolindophenol has been studied to determine its utility as an oxidation-reduction indicator in cellular suspensions.

The first problem that arises in such a study is the determination of the ability of the cells to reduce the indicator. It has been shown by

Gibbs, Cohen and Cannan¹⁰ that 2,6-dichlorophenolindophenol can be readily reduced. They made potentiometric measurements which proved that mixtures of equal amounts of the oxidized and the reduced indicator had the high potential of 0.216 at pH 7.0. Voegtlin, Johnson and Dyer⁷ observed that a related dye, 2,6-dibromophenolindophenol, was rapidly reduced by suspensions of viable normal and viable cancerous tissues under anaerobic conditions but was not reduced by necrotic cancerous tissue. Cohen, Gibbs and Clark¹¹ reported 2,6-dibromophenolindophenol reduced by neutralized suspensions of macerated plant tissue even when a vigorous stream of air was passed through the suspension. Needham and Needham¹² injected this compound into the cytoplasm of marine eggs under aerobic conditions and observed that the indicator was reduced and became colorless. They also showed that the reduced dye could be reoxidized to its original color by potassium ferricyanide. Gibbs, Cohen and Cannan¹⁰ believed it significant that all living cells are able to reduce 2,6-dibromophenolindophenol even in the presence of oxygen. In the present study, suspensions of washed thymic cells of the rat or the rabbit (100 cells per millimicroliter) in phosphate-Ringer solution reduced and kept reduced 2,6-dichlorophenolindophenol, 1:160,000, for many hours.

The second problem that arises is the capacity of the system to resist changes in the oxidation-reduction potential. This problem is analogous to the measurement of the buffering action of a solution at a given pH . Cohen, Chambers and Reznikoff¹³ have shown that amoebas can decolorize 2,6-dichlorophenolindophenol on repeated injections but that ultimately the capacity of the cytoplasm to reduce is overwhelmed. In the present study the capacity of the suspended cells to reduce was measured by adding indicator in varying concentrations to a constant amount of cells. The maximum concentration in which the indicator was completely reduced by the suspended cells was used as a measure of the oxidation-reduction capacity of the cells. For example, washed thymic cells in phosphate-Ringer solution reduced the indicator when its concentration was 1:160,000 but not when its concentration was 1:80,000. In contrast, washed thymic cells plus serum reduced the dye completely even when the concentration was 1:20,000. It is

10 Gibbs, H. D., Cohen, B., and Cannan, R. K., in *Studies on Oxidation-Reduction*, Hygienic Laboratory Bulletin no. 151, United States Public Health Service, 1928, p. 159.

11 Cohen, B., Gibbs, H. D., and Clark, W. M., in *Studies on Oxidation-Reduction*, Hygienic Laboratory Bulletin no. 151, United States Public Health Service, 1928, p. 138.

12 Needham, J., and Needham, D. M. *Proc. Roy. Soc., London*, s. B. **99**: 173, 1926.

13 Cohen, B., Chambers, R., and Reznikoff, P. *J. Physiol.* **11**: 585, 1928.

obvious, then, that the cells plus serum had a much greater reducing capacity than the cells in phosphate-Ringer solution

A third problem in oxidation-reduction is to determine the effect of substrates on the reducing intensity and capacity of the suspended cells. An extensive amount of work has been done by Quastel² on a large number of substrates that enabled washed bacterial cells to reduce methylene blue under anaerobic conditions. No similar studies have been performed on animal cells. Chambers, Beck and Green,⁹ however, observed that ethyl alcohol increased the rate at which the eggs of starfish reduced methylene blue. In the present study, glucose and serum were found to increase the capacity of suspended thymic cells to reduce 2,6-dichlorophenolindophenol. The concentration of glucose in the serum was not sufficient to account for the marked increase in the reducing capacity of the thymic cells. The use of 2,6-dichlorophenolindophenol suggested then that glucose and serum reacted with thymic cells.

Certain agents have an inhibiting instead of a stimulating effect on the reducing intensity or capacity of the cells. Chambers, Beck and Green⁹ found that mercuric oxide in a 1:1,000,000 solution inhibited completely the anaerobic reduction of methylene blue in suspensions of the eggs of starfish. Lead carbonate 1:1,000,000 and ethyl carbamate in a 3 per cent solution retarded the rate of reduction. In the present study, solution of formaldehyde U.S.P. was found to have an immediate and a delayed inhibiting action on the reduction of 2,6-dichlorophenolindophenol. Presumably these inhibitory substances acted by killing the cell, by inhibiting an enzymatic reaction or by combining with a substrate. It seemed that the toxicity of a reagent could be studied by the simple method of determining its inhibition of the power of suspended cells to reduce 2,6-dichlorophenolindophenol.

The use of indicators to measure oxidation-reduction potentials in cellular suspensions has several complications. As Cannan, Cohen and Clark⁸ pointed out, it is important to determine whether the dye itself is toxic or cytotoxic. Chambers, Cohen and Pollack¹⁴ reported that 2,6-dichlorophenolindophenol can penetrate into the echinoderm ovum and that it is toxic. Voegtlin, Johnson and Dyer⁷ studied the toxicity of 2,6-dibromophenolindophenol in the rat on intravenous injection but did not study its toxicity to the cells in suspension. The present study has shown that 2,6-dichlorophenolindophenol has a marked cytotoxic action on washed rabbit thymic cells but not on rat thymic cells in phosphate-Ringer solution.

In a previous study it was observed that thymic cells of the rabbit, but not those of the rat, were rapidly killed on incubation at 45 C. in

14 Chambers, R., Cohen, B., and Pollack, H. J. *Exper. Biol.* 8:1, 1931

the absence of glucose and air. That observation may be compared with the present finding that thymic cells of the rabbit, but not those of the rat, were rapidly killed by 2,6-dichlorophenolindophenol. Presumably both reactions are based on one common factor or deficiency in the thymic cells of the rabbit. What this factor may be would require further investigation.

A second complicating factor in the use of 2,6-dichlorophenolindophenol as an oxidation-reduction indicator is modification of metabolism of the cells by the indicator. Elliot¹⁵ and Elliot and Baker¹⁶ observed that this indicator produced almost complete inhibition of the respiration of tumor tissue in the absence of glucose but accelerated respiration in the presence of glucose. They also found that the indicator inhibited the respiration of kidney, brain, testis and chick embryo both in the presence and in the absence of glucose. The dye had no effect on the glycolysis of tumor. They do not state whether the indicator's inhibition of respiration is due to an inhibiting effect on cellular metabolism or to a lethal action on the cells.

In the present study the effect of the indicator on glycolysis was studied. It was shown that the dye in moderate concentrations stimulated production of acid in the presence of glucose and air. In higher concentrations, however, the dye inhibited glycolysis both under aerobic and under anaerobic conditions. The indicator 2,6-dichlorophenolindophenol has, then, an accelerating or an inhibiting effect depending on the concentration of the dye.

Two conclusions may be drawn. The oxidation-reduction indicator 2,6-dichlorophenolindophenol may be used to determine substances which have a stimulating or an inhibiting effect on cells. It is also useful as a reagent which affects the glycolysis of thymic cells and which has even in dilute solutions a cytotoxic action on washed thymic cells of the rabbit but not on those of the rat. It can be used either as an oxidation-reduction indicator or as a reagent, depending on its concentration, on the time of incubation and on the presence of other substances.

SUMMARY AND CONCLUSIONS

Glucose, mannose and serum increased the capacity of thymic cells to reduce 2,6-dichlorophenolindophenol. Solution of formaldehyde U S P caused an immediate or a delayed inhibition of their power to reduce the dye. Evidently, 2,6-dichlorophenolindophenol as an oxidation-reduction indicator is useful in determining the effects of reagents on cells.

¹⁵ Elliot, K. A. C. *Nature*, London, **134** 254, 1934.

¹⁶ Elliot, K. A. C., and Baker, Z. *Biochem J* **29** 2396, 1935.

By the method of unstained cell counts it was found that the indicator even in a dilution of 1:160,000 had a cytotoxic effect on washed thymic cells of the rabbit but not on those of the rat. The cytotoxic action was inhibited by serum, glucose and mannose.

According to electrometric determinations of hydrogen ion concentrations, the dye in moderate concentrations (1:40,000) caused a considerable increase in aerobic glycolysis and a slight increase in anaerobic glycolysis. In higher concentrations (1:5,000) the indicator inhibited both aerobic and anaerobic glycolysis.

It seems, then, that, in addition to being a useful oxidation-reduction indicator, 2,6-dichlorophenolindophenol is an interesting reagent which affects the metabolism of the cells and which shows a differential reaction between rabbit and rat thymic cells.

CEREBRAL CONCUSSION

Histochemical Demonstration of Nucleases in the Cerebrospinal Fluid

E A SPIEGEL, M D

M SPIEGEL-ADOLF, M D

AND

H T WYCIS, M D

PHILADELPHIA

IN SPECTROPHOTOMETRIC studies of the cerebrospinal fluid in ultraviolet rays, Spiegel-Adolf, Wycis and Spiegel¹ demonstrated, following cerebral concussion, the appearance of substances giving a selective absorption band with a peak at 265 millimicrons. This finding was interpreted as due to the entrance of nucleic acids or their derivatives. If the cerebrospinal fluid was left standing, the selective absorption became weaker and finally disappeared even though the punctate was kept under sterile conditions. It was suspected that this behavior was due to the appearance of enzymatic substances. In order to test this hypothesis, cerebrospinal fluids from normal persons as well as from patients who had sustained cerebral concussion were incubated at 37 C with samples of nucleic acids of animal and of plant origin. Both types of nucleic acids when incubated with the concussion specimens showed a decrease of the selective absorption, while the samples incubated with normal cerebrospinal fluid or saline solution remained unchanged. These experiences seemed to confirm the assumption that enzymatic substances acting on nucleic acids or their derivatives, or both, appear in the cerebrospinal fluid after cerebral concussion.

Spectrophotometric demonstration of such enzymes requires knowledge of a special technic as well as a rather expensive apparatus which is beyond the reach of many routine laboratories. It seemed desirable, therefore, to develop a technic which the average laboratory can easily handle. For this practical reason, as well as from a theoretic point of view, it seemed of interest to study whether an effect similar to that observed in cerebrospinal fluid from patients who had undergone concussion could be observed when the test objects were not nucleic acids.

This investigation was aided by a grant from the John and Mary R. Markle Foundation.

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¹ Spiegel-Adolf, M., Wycis, H. T., and Spiegel, E. *Federation Proc.* 5: 156, 1946.

but nuclear substances within the cells of the central nervous system, in particular the Nissl bodies of the nerve cells. The principle of the method to be described consists in incubating the cerebrospinal fluid under study with paraffin sections of normal spinal cord and to stain these slides by means of the usual Nissl method (with methylene blue, thionine blue or cresyl violet)

In preliminary experiments the effect of normal cerebrospinal fluid on the anterior horn cells of the cat's spinal cord was studied. To our surprise, such cerebrospinal fluid produced tigrolysis in the anterior horn cells. The specimens of cerebrospinal fluid were used immediately after lumbar puncture and were kept under sterile conditions. Further experiments showed that even Ringer's solution when incubated for several hours at 37 C with sections of cats' spinal cords was able to bring the Nissl bodies into solution. It occurred to us that old experiments of Held² had pointed to the importance of the acidity of the solution for the solubility of the Nissl bodies. Therefore, by adding buffer solutions to Ringer's solution or to the cerebrospinal fluid under study, the hydrogen ion concentration of the solution or fluid acting on the sections of spinal cord was varied systematically. It was found that solution or fluid with a p_H between 2.0 and 4.1 did not affect the Nissl bodies significantly when incubated at 37 C for four to five hours. When solutions of a p_H of 4.6 or higher were used, the Nissl bodies of the anterior horn cells were much finer than normal. When the p_H of the solution was above 6.0, definite signs of solution of the Nissl bodies appeared. These experiments seemed to indicate that at the hydrogen ion concentration of the normal cerebrospinal fluid the Nissl bodies go into solution after several hours' incubation at 37 C. These experiences may also shed some light on the old question whether the Nissl bodies are preformed in the living cells (Bielschowsky³). Apparently, at the normal hydrogen ion concentrations of the body fluids or cell fluids the nucleoproteins of the nerve cells are kept in solution, and only when postmortem changes cause an accumulation of acids in the central nervous system are the nucleoproteins precipitated—and the results are the Nissl bodies. It proved important, therefore, to acidify the cerebrospinal fluid before it was incubated with the spinal cord slides. As a rule, a buffer solution with a p_H of 4.05 was added to the cerebrospinal fluid. Thus the following method was finally adopted.

The test objects are, as a rule, the spinal cords of cats. The cords are fixed in 70 per cent alcohol. The blocks are embedded in paraffin by the usual method,

2 Held, H. Arch f Anat u Physiol (Anat Abt.), 1895, p. 396

3 Bielschowsky, M. Morphologie der Ganglienzellen, in von Mollendorff, W. Handbuch der mikroskopischen Anatomie des Menschen, Berlin, Julius Springer, 1928, vol. 4, p. 8

and paraffin sections 5 to 10 microns in thickness are cut. Two pairs of sections of the cat's cord are placed on a slide. After the sections are deparaffinized, each pair is surrounded by a square frame corresponding to the size of a cover glass of 18 sq mm. Such slides may be kept in stock for testing specimens of cerebrospinal fluid as soon as they are brought from the operating room.⁴ It has been found that 0.25 to 0.50 cc of cerebrospinal fluid is sufficient for such a test. As a rule, 0.45 cc of the fluid is mixed with 0.05 cc of an acetate buffer⁵ of p_H 4.05 in a sterile tuberculin syringe. About 0.2 to 0.25 cc is necessary to fill the area within the paraffin square. After this amount of fluid has been deposited within the paraffin frame, it is covered by a clean cover glass, and the edges between the cover glass and the frame are sealed with paraffin so that they are air tight. It is important that the fluid cannot evaporate when the slide is put in the incubator and that no air bubbles are left within the sealed area, since changes in the concentration of the fluid may affect the nerve cells. Usually one pair of sections is covered with cerebrospinal fluid and the other with Ringer's solution, which is also acidified with the buffer solution in the same proportion, 9 to 1. The same procedure may be repeated on the second slide, or on the second slide only one square is covered with cerebrospinal fluid and the other square left without fluid. The slides are then put in an incubator at 37 C for four hours.⁶ At the end of this period the cover glasses and the paraffin frames are removed, and the slides are washed in distilled water and subjected to the routine staining with 0.1 per cent thionine solution. On differentiation of the slides with alcohol, it is important to check the progress of the differentiation under the microscope repeatedly. If the cerebrospinal fluid has produced a high degree of tigrolysis, the differentiation should not be too intense, so that the cytoplasm of the cells is still clearly visible. In such a case the control sections which were under the influence of Ringer's solution or were not subjected to any fluid at all are, of course, slightly overstained. In any case it is important to treat both pairs of sections, those under the influence of cerebrospinal fluid and the control sections, in exactly the same manner.

In figure 1 the parts lettered *a* show anterior horn cells of the spinal cord of a normal cat which were subjected to the cerebrospinal fluid of a patient with cerebral concussion. The interval between the trauma and the lumbar puncture was three days. A tigroid structure is hardly visible, the major part of the cytoplasm is only faintly stained, appearing homogeneous or slightly granulated, while its remaining part shows a more or less homogeneous, darkly stained mass. The peripheral part of the cell usually is more affected than the central part. Control sec-

4 As a rule, the specimens were studied within a few hours after tapping. Control experiments showed that specimens preserved in an ice box for a few days under sterile conditions can still be used for this enzyme test, while a specimen standing in the ice box for over a week may affect the anterior horn cells on incubation even if this specimen is taken from a patient with a normal central nervous system.

5 The buffer is prepared by mixing 150 cc of molar acetic acid with 30 cc of molar sodium acetate.

6 In some of our earlier experiments we kept the slides for four to five hours at 37 C and for seventeen to nineteen hours at room temperature (23-25 C).

tions (fig 1 *b* and 1 *c*)—one kept in the incubator without the addition of any fluid and one subjected to the influence of Ringer's solution—are overstained for the reasons mentioned in the foregoing paragraph

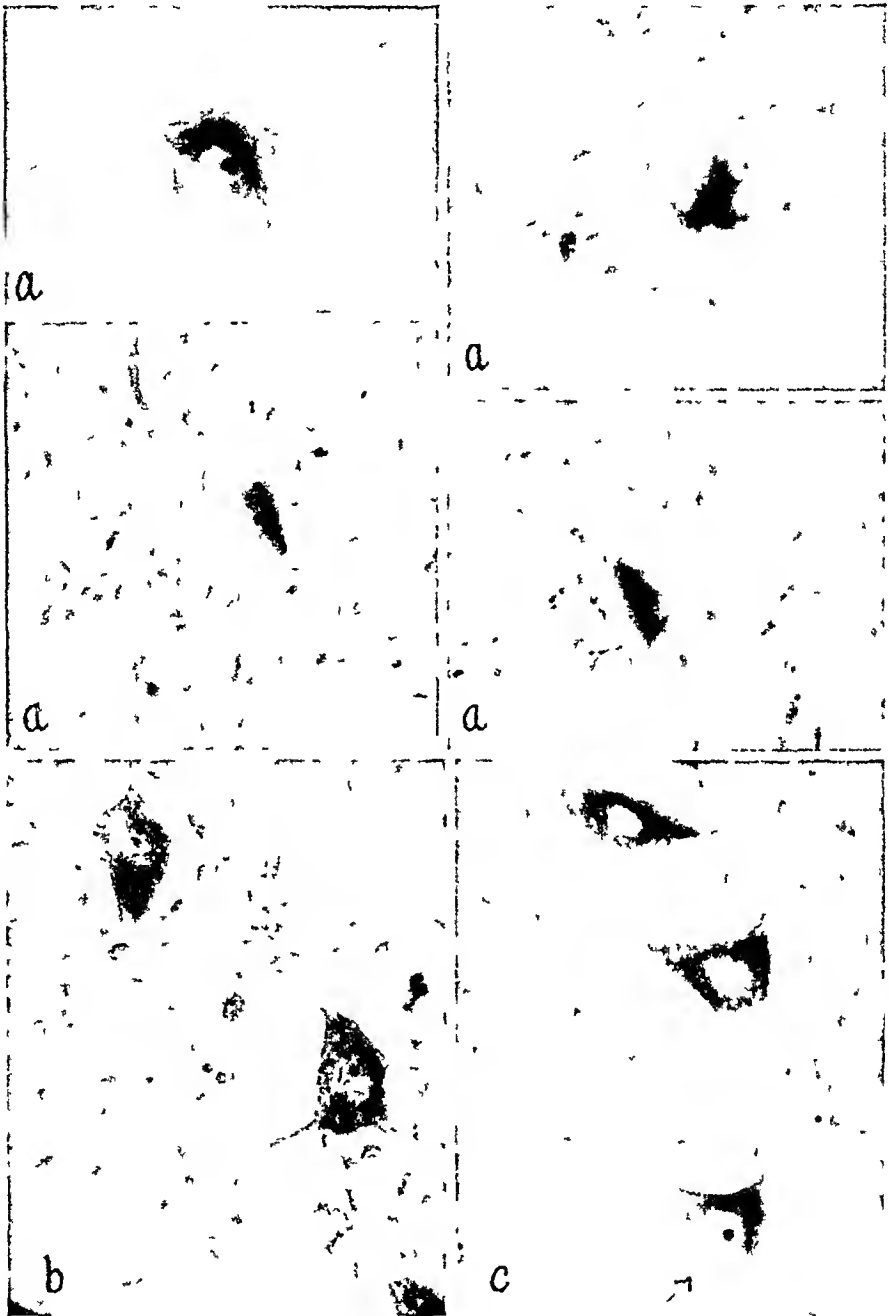


Fig 1—Anterior horn cells in sections from the spinal cord of a normal cat. The sections were stained with toluidine blue after incubation (*a*) with cerebrospinal fluid from patient W, who had sustained cerebral concussion, (*b*) without addition of any fluid and (*c*) with Ringer's solution. The interval between the cerebral trauma and the lumbar puncture was three days. For further details see the text.

But they show clearly that in the large majority of the cells the Nissl bodies are well preserved. Only occasionally (arrow in fig 1 *c*) a cell shows an area in its periphery where the tigroid bodies are only faintly stained or are not demonstrable. Figure 2 *a* represents the effect of the cerebrospinal fluid from another patient with concussion, which was tapped twelve days after the cerebral trauma. The solution of the nucleoproteins is still more advanced, probably because the specimens were subjected to the influence of the fluid for a longer time (five hours at 37 C, seventeen hours at 25 C). Practically all the anterior horn cells fail to show Nissl bodies, the cytoplasm represents a homogeneous mass, sometimes traversed by fissures, only the nucleolus may be demonstrable. In contradistinction the glia nuclei seem rather well preserved. The

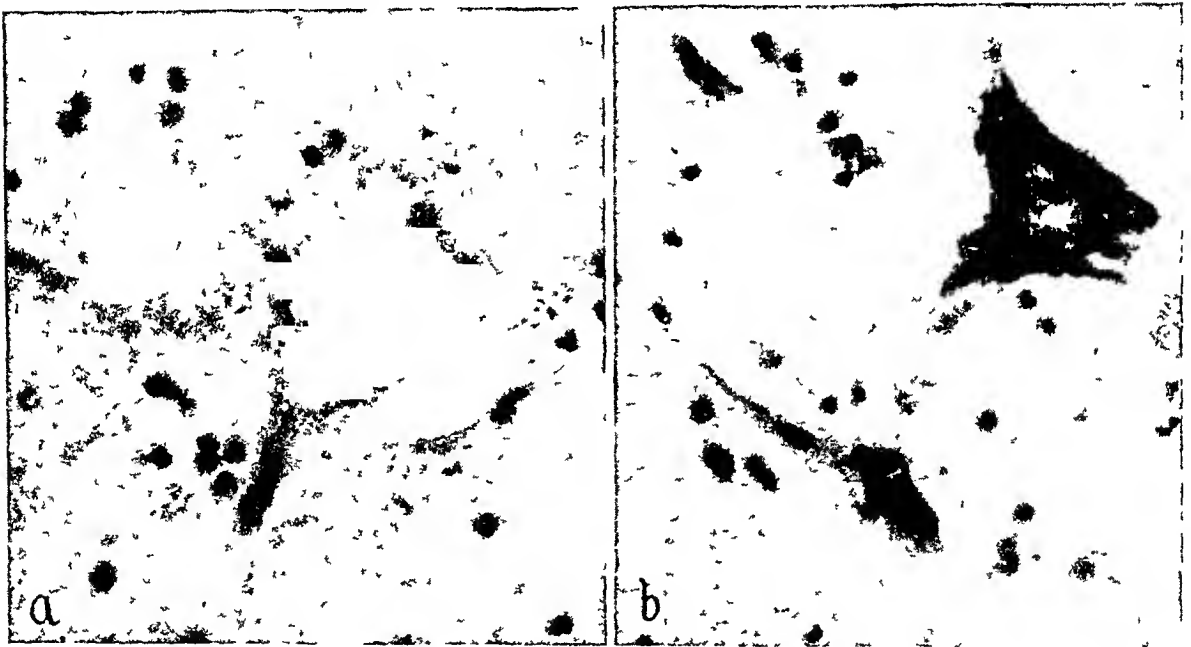


Fig 2—Anterior horn cells in sections from the spinal cord of a normal cat which were stained with cresyl violet after incubation with (*a*) cerebrospinal fluid from patient C, who had undergone cerebral concussion, and (*b*) Ringer's solution. The interval between the accident and the lumbar puncture was twelve days.

intercellular tissue is more darkly stained than that in the control sections, which were subjected to Ringer's solution (fig 2 *b*), perhaps because the dissolved nucleoproteins or their cleavage products diffused into the pericellular tissue. It should be emphasized that the tigroid bodies of single cells may be affected also if buffered Ringer's solution or cerebrospinal fluid of patients with apparently normal central nervous system is incubated with sections of cats' cords. Therefore the conclusion that one deals with nucleases or similar enzymatic substances seems warranted only if the cerebrospinal fluid is able to produce tigrolysis in all or in the majority of the anterior horn cells studied.

While figures 1 and 2 represent cases in which the cerebrospinal fluid was tapped within a few days after the trauma, figure 3 shows the influence of a cerebrospinal fluid that was obtained one-half year after the accident that produced the cerebral concussion. This fluid was no longer able, on incubation, to affect the cells of the cat's anterior horn.

In a parallel study, these cerebrospinal fluids were studied by spectrophotometry in ultraviolet rays, and it was found that the fluids producing tigrolysis were able to decrease the characteristic absorption power of nucleic acids, while the fluids which left the anterior horn cells intact also failed to affect the nucleic acids. Thus the histochemical and the spectrophotometric method confirmed each other.

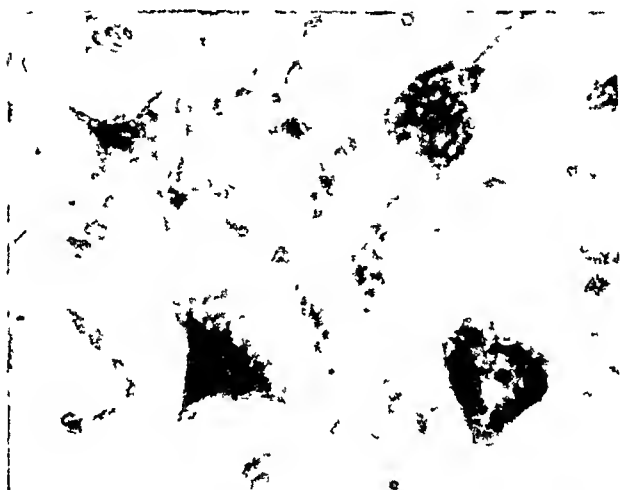


Fig 3—Anterior horn cells in a section from the spinal cord of a normal cat. This section was incubated with cerebrospinal fluid of patient D (cerebral concussion) and then stained with toluidine blue. The interval between the accident and the lumbar puncture was one-half year.

The demonstration that the cerebrospinal fluid of patients with cerebral concussion contains substances able to produce solution or a breakdown of Nissl bodies seems of interest not only from a practical but also from a theoretic point of view. If such substances diffuse from the central nervous system into the cerebrospinal fluid, it seems not unreasonable to suspect that they play an important role in the genesis of the chromatolytic changes observed after concussion.

7 The chemical nature of the enzymes taking part in the mechanism of tigrolysis remains to be studied. It seems probable that they are not only nucleases but also desaminases. The absorption band at 265 millimicrons found by spectrophotometric study of cerebrospinal fluid from patients who had undergone cerebral concussion can be caused not only by nucleic acids but also by some of their cleavage products such as pyrimidine bases. It has been pointed out that the cerebrospinal fluid of such patients is able to induce a diminution of the specific absorption band. This observation indicates that the substances diffusing

SUMMARY

Incubation of buffered specimens of cerebrospinal fluid from patients who had undergone cerebral concussion with sections of cats' cords produced tigrolysis in the anterior horn cells, while similar treatment of the sections with buffered specimens of cerebrospinal fluid from normal persons or with buffered Ringer's solution failed to produce such an effect

The findings point to the importance of enzymatic substances in the genesis of chromatolysis following cerebral concussion

The nucleoproteins of the nerve cells go into solution at the hydrogen ion concentration prevailing in the normal central nervous system *in vivo*. When sections of spinal cords are incubated with various fluids, these fluids must be acidified for demonstration of Nissl bodies

from the central nervous system into the subarachnoid space after concussion are not only nucleases causing a breakdown of nucleic acids but also substances acting on their nitrogen-containing decomposition products (desaminases, according to J. B. Summer and G. F. Somers [Chemistry and Methods of Enzymes, New York, Academic Press, 1943, p. 103])

EXPERIMENTAL MEDIONECROSIS OF THE AORTA

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DISSEMINATED medionecrosis of the aorta of man, a common cause of spontaneous rupture of the ascending aorta and of dissecting aneurysm, has been carefully studied by many workers¹ The first systematic pathologic reports were made by Gsell^{1a} and Erdheim^{1b} One of the best reports is that of Rottino and his co-workers^{1c,d} Erdheim gave this entity the name "medionecrosis cystica idiopathica" because of the necrosis present in the muscular and the elastic tissue and the mucoid-cystic degeneration of the media

Experimentally, necrotic and degenerative changes of the media of the abdominal aorta and the peripheral arteries have been observed in rabbits by many investigators, following crushing of the vascular wall, sheating of the vascular wall in paraffin, dissecting off of the adventitia and applying of acids and thermocautery to the outside of the vascular wall² One of the most careful and elaborate studies was

* Fellow of the Dazian Foundation

This investigation was aided by the A D Nast Fund for Cardiovascular Research

From the Cardiovascular Department, Michael Reese Hospital This department is supported in part by the Michael Reese Research Foundation

1 (a) Gsell, O Virchows Arch f path Anat. **270** 1, 1928 (b) Erdheim, J ibid **273** 454, 1929, **276** 187, 1930 (c) Rottino, A Arch Path **27** 320, 1939, **28** 1 and 377, 1939 (d) Rottino, A, and Poppiti, R ibid **36** 201, 1943 (e) Cellina, M Virchows Arch f path Anat **280** 65, 1931 (f) Weise, W Beitr z path Anat u z allg Path **93** 238, 1934 (g) Zimmerman Klin Wchnschr **14** 500, 1935 (h) Wolff, K Virchows Arch f path Anat **289** 1, 1933 (i) Eck, H Frankfurt Ztschr f Path **52** 276, 1938 (j) Harrison, F F Arch Path **27** 742, 1939 (k) Wenger, F ibid **36** 253, 1943 (l) Levinson, B Virchows Arch f path Anat **282** 1, 1932 (m) Roberts, J T Am Heart J **18** 188, 1939 (n) Castellaneta, V Gior di med mil **80** 282, 1932 (o) Orsos, F Verhandl d deutsch path Gesellsch **26** 365, 1931 (p) Oppenheimer, R Virchows Arch f path Anat **181** 382, 1905 (q) Moritz, A R Am J Path **13** 679, 1937 (r) Neubuerger, K Ztschr f Kreislaufforsch **24** 169, 1932 (s) Gunther, G W Verhandl d deutsch path Gesellsch **31** 363, 1938

2 (a) Malkoff, G M Beitr z path Anat u z allg Path **25** 431, 1899 (b) d'Anna, E Policlinico **4** 62, 1897 (c) Lange, F Virchows Arch f path Anat **248** 463, 1924 (d) Andriewitsch Inaug Dissert, St Petersburg, 1901 (e) Fabris, A Virchows Arch f path Anat **165** 439, 1901 (f) Sumikawa, P Beitr z path Anat u z allg Path **34** 242, 1903 (g) Hildebrandt, F Experimentell erzeugte lokale Atherosklerose und ihre Beziehungen zur

that of Lange^{2c} Many agents, such as parenterally injected epinephrine hydrochloride, produced medionecrotic lesions in rabbits' arteries³

In dogs, medionecrotic lesions could not be produced until recently, when Hueper and Ichniowski⁴ reproduced such lesions in the aorta and the large arteries by injecting lethal and sublethal doses of histamine dihydrochloride The lesions resembled closely those in the human

Niere, Inaug Dissert, Heidelberg, J Hoerning, 1912 (h) Jores, L, cited by Ssolowjew^{2m} (i) Borst and Enderlen Deutsche Ztschr f Chir **99** 54, 1909 (j) Ziegler, E Verhandl d deutsch path Gesellsch **1** 85, 1898, Zentralbl f allg Path u path Anat **9** 844, 1898 (k) Jaffe, R W, Willis, D, and Bashem, A Zentralbl f allg Path u path Anat **44** 241, 1929 (l) Malyschew, B F Virchows Arch f path Anat **272** 727, 1929 (m) Ssolowjew, A Beitr z path Anat u z allg Path **83** 485, 1929

3 (a) Josue, O Compt rend Soc de biol **57** 539, 1904, Arch gen de med **28** 51, 1904, J de physiol et de path gén **7** 690, 1905 (b) Fleischer Ergebn d allg Path u path Anat **9** 559, 1909 (c) Thorel, C Ergebn d allg Path u path Anat **9** 559, 1904 (d) Baylac, I Compt rend Soc de biol **58** 935, 1906 (e) Falk, F Verhandl d Kong f inn Med **24** 451, 1907, Ztschr f exper Path u Therap **4** 360, 1907 (f) Fischer, B Munchen med Wchnschr **66** 61, 1919, Ztschr f Psychiat **62** 241, 1904 (g) Lissauer, M Berl klin Wchnschr **42** 675, 1905 (h) Klieneberger, C Zentralbl f inn Med **28** 273, 1907 (i) Erb, W, Jr Arch f exper Path u Pharmakol **53** 173, 1905 (j) Orlowsky Russk vrach **4** 1443, 1905 (k) Schirokogoroff, J J Virchows Arch f path Anat **191** 482, 1908 (l) Kalamkarow, I G Russk vrach **6** 366, 1907 (m) Stief, A, and Tokay, L J Nerv & Ment. Dis **81** 633, 1935 (n) Waterman, N Virchows Arch f path Anat **191** 202, 1908 (o) Heusner, R Beitr z path Anat u z allg Path **58** 89, 1914 (p) Ziegler, K Z ibid **38** 229, 1905 (q) Rzetkowski, K Gaz lek **24** 691 and 709, 1904 (r) Scheidemandel, E Virchows Arch f path Anat **181** 363, 1905 (s) Lortat-Jacob and Sabareanu Compt rend Soc de biol **57** 444, 1904 (t) Pic, A, and Bonnamour, S ibid **58** 219, 1905 (u) Baylac, I ibid **57** 640, 1904 (v) Loeper, M Presse méd **14** 233, 1906 (w) Hedinger, E Cor-Bl f schweiz Aerzte **35** 634, 1905 (x) Braun, L Munchen med Wchnschr **52** 539, 1908 (y) von Koranyi, A Deutsche med Wchnschr **32** 679, 1906, **33** 191, 1907 (z) Torri, O Centralbl f allg Path u path Anat **17** 319, 1906 (a') d'Amato, L Berl klin Wchnschr **43** 1100 and 1131, 1906, München med Wchnschr **53** 757, 1906 (b') Trachtenberg, M A Charkow M J **3** 150 and 468, 1907 (c') Aschoff, L, and Cohn Verhandl d deutsch path Gesellsch **12** 131, 1908 (d') Iwanowsky, B D Virchows Arch f path Anat **297** 100, 1936 (e') Handelsman, J Ueber Suprarenininjektionen bei Kaninchen nebst Einleitung über Nebennierenveränderungen bei Arteriosklerose, Inaug Dissert, Berlin, E Ebering, 1906 (f') Kaiserling, C Berl klin Wchnschr **44** 29, 1907 (g') Pearce, R M, and Baldauf, L K Am J M Sc **132** 737, 1906 (h') Loeb, L, and Fleischer, M S Am J M Sc **133** 903, 1907 (i') Cummins, W T, and Stout, P S Pennsylvania M Bull **19** 101, 1906 (j') Muhlmann, M, and Sehmel, J Beitr z path Anat u z allg Path **81** 211, 1928 (k') Lange, F Virchows Arch f path Anat **248** 463, 1924 (l') Miller, J L Am J M Sc **133** 593, 1907 (m') Kubo, I Folia pharmacol japon **31** 63, 1941 (n') Otto, L Virchows Arch f path Anat **203** 352, 1911

4 Hueper, W C, and Ichniowski, C T Am J Path **20** 211, 1944, J Pharmacol & Exper Therap **78** 127, 1943

aorta described by Gsell and Eidheim and those which follow burn shock⁶

The formation of aneurysms has been observed in rabbits after injection of epinephrine hydrochloride⁶ There are also reports of aneurysm formation in cholesterol-fed rabbits⁷ Leary and Weiss⁸ observed a dissecting aneurysm of the aorta arising in an atheromatous ulcer in a cholesterol-fed rabbit, which lived for three years However, there are no reports of spontaneous rupture or dissecting aneurysm of the aorta due to necrotic changes of the media in dogs

My associates and I became interested in the possibility of reproducing these necrotic lesions in dogs by interfering with the vascularization of the aorta Since medionecrotic lesions of the aorta and the consequent spontaneous rupture and dissecting aneurysms usually are found in the ascending aorta, we concentrated our studies on this portion of the aorta

METHOD

In 7 dogs the adventitia of the ascending aorta was coagulated by means of a specially devised simple cautery brought to a red glow over the flame of a Bunsen burner Dogs weighing 20 to 30 pounds (9 to 13.5 Kg) were used During anesthesia (pentobarbital sodium, 25 mg per kilogram) and artificial respiration, the chest was opened aseptically by a 4 to 7 cm incision in the third or fourth right intercostal space beginning medially 2 cm from the sternal margin The pericardium was incised, the ascending aorta exposed and the fat pad carefully resected An area of 2 sq cm of the adventitia was then coagulated in the region in which our previous studies had shown that the vasa arising from the right coronary artery anastomose most frequently with those from the left coronary artery and those from the brachiocephalic vessels of the arch The thorax was then closed in layers with cotton thread, the pericardial sac being left open, and the pneumothorax was relieved The dogs were permitted to recover from the anesthesia They were killed after one, two or six weeks

Two other dogs, previously made hypertensive by daily injections of desoxycorticosterone acetate while being permitted to drink 1 per cent sodium chloride solution ad libitum,⁹ were used in this study In both animals the blood pressure reached 225 mm of mercury systolic and 125 mm diastolic One was used as a control, the other underwent cauterization of the aorta and was killed six weeks later

The changes in the aorta in the first six to twelve hours after coagulation were studied in 4 dogs In these animals, the chest was widely opened, and artificial respiration and anesthesia were maintained until they were killed

5 Zinck, K. H. *Klin Wchnschr* 17 278, 1938 Meesen, H. *Klin Wchnschr* 17 1635, 1938, *Beitr z path Anat u z allg Path* 105 432, 1941, 102 191, 1939, 99 329, 1937 Günther¹⁸

6 Fischer²² Erb²¹ Schirokogoroff²³ Kaiserling²⁴

7 Liebig, H. *Klin Wchnschr* 8 1516, 1929, 10 475, 1931, 20 538, 1941, *Arch f exper Path u Pharmakol* 159 265 and 359, 1931, 175 409, 1934 Wesselkin, N. W. *Virchows Arch f path Anat* 212 225, 1913

8 Leary, T., and Weiss, S. *Arch Path* 29 665, 1940

9 Rodbard, S., and Freed, S. C. *Endocrinology* 30 365, 1942

Autopsies were made on all the animals studied. The vasa of the aorta were injected, the aorta was then dissected, and roentgenograms were taken in the manner previously described. Microscopic sections of the aortas fixed in solution of formaldehyde U S P were prepared and stained with hematoxylin and eosin, orcein, Masson and Van Gieson stains.

RESULTS

All the dogs survived the operation. Those permitted to come out of anesthesia behaved normally within a few hours after the operation with 1 exception. The exceptional animal refused food and water and was apathetic on the third post-operative day, it recovered in the next two days and behaved normally until killed fourteen days after the operation.

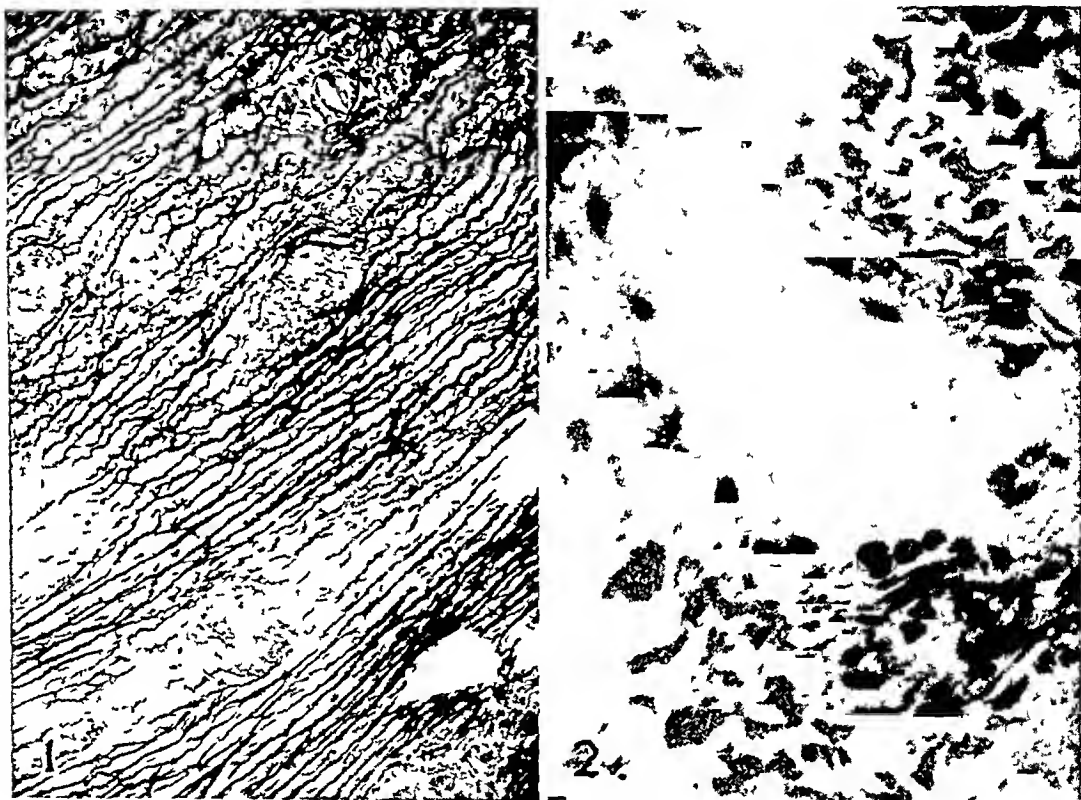


Fig 1—Outer and middle third of the aortic media showing diffuse necrosis. There are loss of nuclei, homogenization of the media and stretching and swelling of the elastic fibers. The disintegration of muscular tissue is accompanied by swelling, fusion, fragmentation and clumping of elastic fibers. There are areas of liquefaction with cyst formation, with or without mucoid material. Hematoxylin-eosin stain, $\times 800$.

Fig 2—Middle third of the media showing cyst filled with mucoid material. Hematoxylin-eosin stain, $\times 4000$.

Aside from the adventitial coagulation, no gross alterations were seen in the aorta at the end of six to twelve hours. Microscopically, however, small hemorrhages were visible in the outer third of the media. The inner third of the media showed marked edema with separation of the elastic and muscle fibers (fig 1). The intima showed no changes. In no instance could necrosis or cellular infiltration be seen in the media.

At the end of one to six weeks the pericardium and the right auricle were more or less adherent to the coagulated part of the aortic adventitia, the degree of adherence being more pronounced the longer the dogs survived.

One week after the operation the aorta on section showed yellowish brown areas in the media. These were noted not only beneath the coagulated adventitia but also beneath normal adventitia as far away from the coagulation as 1 to 2 cm. The intima showed no gross changes.

Two to six weeks postoperatively the gross changes were similar in location but the yellowish brown areas were partially replaced by grayish areas. The extent of the grayish areas increased the longer the dog survived. At the end of six weeks these grayish areas extended into the intima.

The dog which had been indisposed postoperatively showed at necropsy, at the end of two weeks, a bulging of the adherent area which was moderately soft to touch and extended the whole length of the ascending aorta. Careful examination revealed a rupture of the aorta, 0.5 mm in length, 2 cm above the aortic cusps, which had caused a dissection up to the arch. This dissecting aneurysm extended a short distance through the media and then between the media and the adventitia.

In the hypertensive animal (given desoxycorticosterone acetate) a large intimal plaque was found six weeks postoperatively. This plaque occupied the whole aortic circumference and measured approximately 4 sq cm. The aorta in this region was inelastic and felt thinner. No yellowish brown areas were seen in this dog.

The microscopic observations were as follows. One week postoperatively the outer and middle thirds of the media were diffusely necrotic. This was evidenced by loss of nuclei, homogenization of the media and stretching and swelling of the elastic fibers. The disintegration of muscular tissue was accompanied by swelling, fusion, fragmentation and clumping of elastic fibers (fig 1). Areas of liquefaction with cyst formation, with or without mucoid material, were observed (figs 2 and 3). These necrotic areas appeared not only beneath the coagulated adventitia but also beneath normal adventitia (fig 4). They were noted also in the middle part of the media, surrounded by normal media (figs 4 and 5). On the other hand, normal media was noted below the coagulated adventitia. With the latter one could observe blood vessels filled with contrast dye in the media. These blood vessels must have derived their supply from the collateral circulation via the extensive anastomotic channels,¹⁰ since the vessels of the adjacent adventitia had been destroyed by the coagulation. In every case of diffuse necrosis the blood vessels in the necrotic areas were not injectable. A few hemorrhages could be seen filling spaces which had undergone liquefaction, and these were located near the line of demarcation between necrotic and normal media. The inner third of the media showed focal necrosis with a predominance of cystic changes, but revealed no diffuse homogenization. Fragmentation, granulation and clumping of elastic fibers in these areas were predominant. The focal lesions showed a distribution similar to that observed in the middle and outer media. In many instances young collagenous fibers started to replace the necrotic tissue and the albuminoid material of the cysts. No instance of cellular infiltration of the media was observed. Except for 1 dog with two areas of slight intimal proliferation, no pathologic alteration of the intima was noted (fig 5).

In the second week the medial changes were similar to those noted in the first week with some additions. However, no hemorrhages were observed. The additional changes consisted of (a) much more marked breakdown of elastic

10 Schlichter, J. G. *Am Heart J*, to be published.

fibers, (b) replacement of cystic and necrotic areas by young fibrous tissue, (c) increase of the cystic areas in the inner third of the media, (d) appearance of fibrous tissue growing into the media from the adventitia and (e) more marked intimal proliferation above the necrotic areas

One of the dogs which, as previously mentioned, showed rupture of the aortic wall had necrosis of the whole aortic wall and homogenization in this area. The intima around the rupture showed thickening and marked proliferation (fig 7). The rupture extended from the intima to the media and had dissected between the latter and the adventitia (fig 8). Fibrous tissue was seen growing into the rupture from the adventitia, and organization of thrombotic material was noted

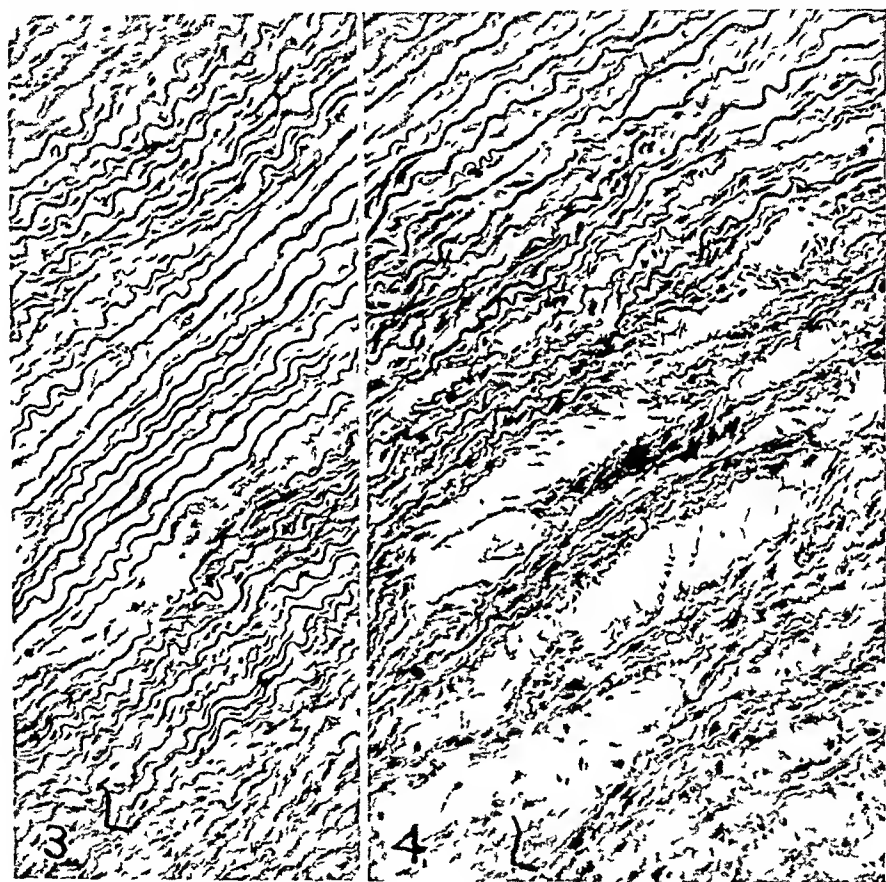


Fig 3—Middle third of the media showing necrosis of the muscular tissue with stretching and swelling of elastic fibers. The necrotic area is surrounded by normal media and, beneath, normal adventitia. Hematoxylin-eosin stain, $\times 132$

Fig 4—Necrosis of the outer third of the media is present, and cystic and necrotic areas are being replaced by fibrous tissue. Hematoxylin-eosin stain, $\times 144$

Hyalinization of the walls of some vasa with luminal thrombosis was observed in the adventitia at a spot next to the coagulation.

In the fourth to sixth week postoperatively, the replacement of necrotic tissue by collagenous and fibrous tissue was pronounced, and intimal proliferation was marked above the necrotic areas. The necrotic areas were replaced more and more by fibrous tissue, which formed an irregular meshwork in the middle and inner thirds of the media (fig 9). The cystic areas were replaced or filled by

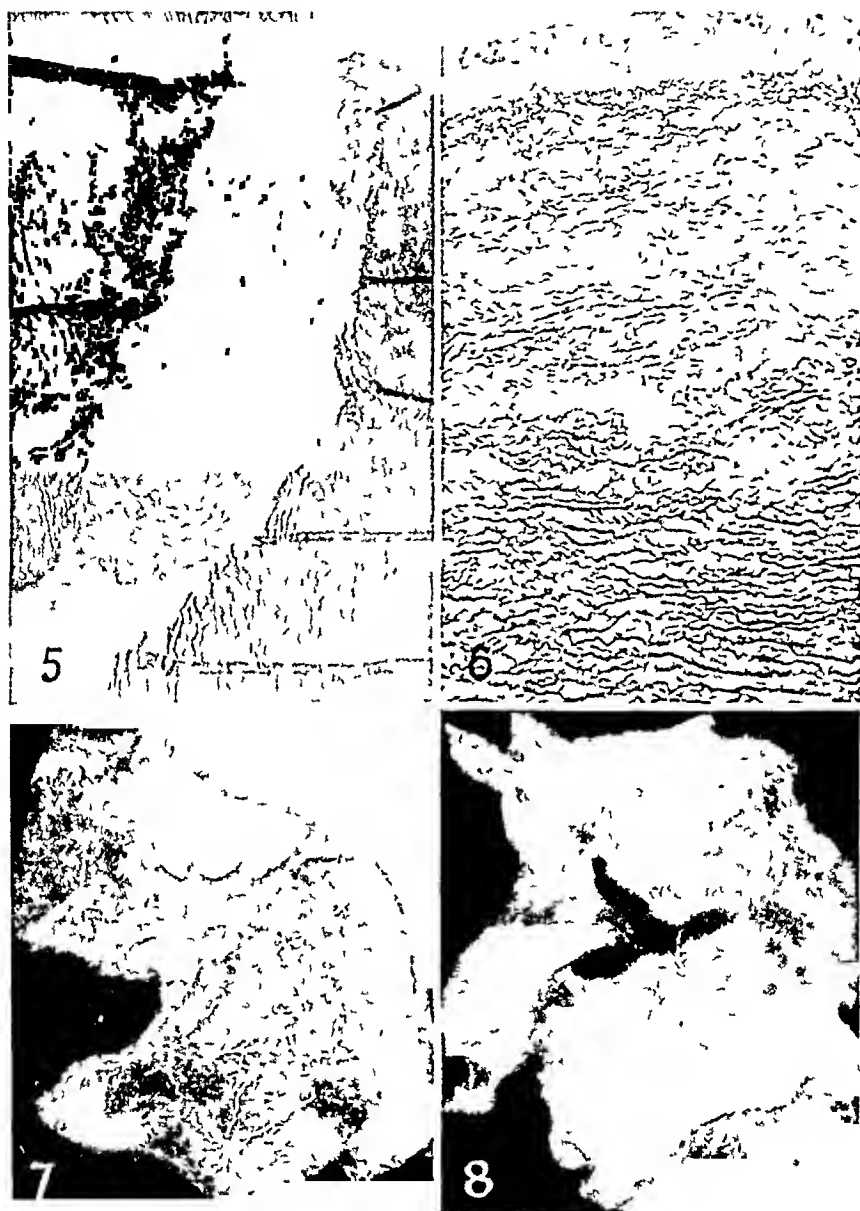


Fig 5—Necrosis of the whole aortic wall with homogenization of the media. There is a rupture of the aortic wall. The intima around the rupture shows thickening and proliferation. Fibrous tissue is growing into the rupture from the adventitia, and organizing thrombotic material can be noted. Hematoxylin-eosin stain, $\times 16$.

Fig 6—Extensive proliferation of the intima in the hypertensive dog. Hematoxylin-eosin stain, $\times 616$.

Fig 7—Marked increase and irregularity of the vascularity of the coagulated aorta of the hypertensive dog six weeks after coagulation. Note the presence of a vessel arising directly from the intima, proved by histologic examination.

Fig 8—The injected ruptured aorta shows no vessels in the coagulated area, and there are no anastomoses between the right and the left coronary artery. The arcuate branches of the coronary arteries are also severed.

NOTE.—This photograph, in the course of reproduction, was turned. What now is the left side should be the top.

connective tissue (fig 10) Hyalinization of small areas of the inner third of the media and of the intima was observed, and these areas protruded into the lumen of the aorta. The fibrous tissue growing into the adventitia and the outer third of the media was richly vascularized, the vascularity being much more pronounced than that observed in normal dogs. Nevertheless, no filling of medial vessels could be seen in these areas.

The changes in the hypertensive dog were the same except that the proliferation of the intima (fig 11) was much more marked and a vessel opening directly from the lumen of the aorta was observed in the area above the medial alterations. Incidentally, the control, hypertensive dog revealed no abnormal changes.

Roentgenograms of the aorta revealed that anastomoses between vasa arising from the left and from the right coronary artery and vasa from the vessels of the aortic arch were still present (fig 12). Because of the rich anastomoses, the coagulation had not completely interrupted the blood supply. Six weeks after coagulation of the adventitia, the vascularity of the aorta appeared as a dense anastomotic network in the coagulated area. The intimal vessel direct from the lumen of the aorta in the hypertensive dog has already been mentioned (fig 13).

In the dog with aortic rupture, unlike the others, complete absence of vascularity was found in the area which ruptured. No anastomoses or collaterals appeared to exist in this animal between the vasa arising from the left and the right coronary artery or between them and the arcuate branches of the coronary arteries, which could supply the ruptured area (fig 14).

COMMENT

These studies have revealed some of the factors involved in the changes in the structure of the aortic wall when its vascularity is experimentally interfered with. The presence of an extensive collateral circulation minimizes the degenerative process following interference with the adventitial vascularization or, at least, retards it. Because of the slowing up of the degenerative processes, mucoid degeneration and cysts form in the outer and middle thirds of the media. Necrosis of the whole wall develops only when the circulation of the areas is completely severed and the supply through stomas is minimal. Such extensive necrosis can lead to rupture, and the rupture can dissect slowly to form a dissecting aneurysm. This actually occurred in a dog observed in this study. On the basis of the clinical findings in this dog it would appear that the rupture developed on the third postoperative day. The tendency to heal was evidenced by the presence of fibrous tissue growing into the rupture and by the organization of thrombotic material. Cases of healed rupture of the human aorta have been reported (1^m).

No instance of cellular infiltration of the media was found. The necrotic and the cystic areas were replaced by collagenous and fibrous tissue, and new vascularization of the adventitia appeared. This process seemed to be complete by the fourth to sixth postoperative week. The revascularization is more abundant than that in normal dogs, although it is irregular in its extent. One could not demonstrate any new medial network in these areas, at least of vessels injectable with the dye. Smaller

vessels, below 10 microns, were seen microscopically. In 1 case, a vessel opening directly from the lumen of the aorta was seen filled with dye. It is not possible to state whether or not this vessel was newly developed or had existed before operation. In several instances it was apparent that the presence of large vessels arising from the intima, or of collaterals and anastomoses from uncoagulated adventitia, tended to prevent the development of medial necrosis. On the other hand, necrosis developed above areas of normal adventitia if these areas belonged to the supply of coagulated vessels and had insufficient collaterals.

Some of the animals in which the adventitial coagulation was superficial in areas showed hyalinization with thrombosis of vasa vasorum.

The hypertensive dog showed more extensive proliferation of the intima than the other dogs and a few areas of hyalinization in the intima and the outer third of the media. The wall below the coagulated adventitia could be considered a small true aneurysm, it was thinner, inelastic and had marked fibrous replacement. Desoxycorticosterone acetate was used in this dog, and it has been reported to cause medionecrosis. However, the control dog to which this drug was given, but which had no adventitial necrosis, showed nothing unusual in its aorta. Hypertension is important in the after-effects of medionecrosis. It weakens the aortic wall involved by this lesion and leads to a greater tendency toward aneurysmal dilatation. By putting a greater strain on the aorta, it leads to a greater chance of rupture of the previously necrotic wall. Even in normotensive subjects, however, there is enough rapid and marked fluctuation in blood pressure under conditions of emotional or physical strain or with coughing to lead to rupture of a necrotic aortic wall (Schlichter¹¹).

Heuper and Ichmowski¹ reproduced degenerative and cystic changes in the dog's aorta by subjecting the animals to lethal or sublethal histamine shock. Dogs treated with repeated intravenous injections of excessive doses of epinephrine hydrochloride did not show arterial lesions. Otto^{2a} found that administration of epinephrine extending for long periods led to fibrous thickening of the intima and some medial degeneration. The rabbit is a much more suitable animal for the reproduction of medionecrotic changes resembling those in man. Many endogenous and exogenous agents, epinephrine being the most commonly employed substance, have produced medionecrotic lesions in the aorta and other arteries of the rabbit. Hueper,¹² in his recent review, stated that it is generally conceded that acute epinephrine-

11 Schlichter, J. G. *Beitrag zu den Aneurysmen und Rupturen des Herzen*, Thesis, Lausanne, University of Lausanne, 1940.

12 Hueper, W. C. *Arch Path* 38 162, 245 and 350, 1944, 39 51, 117 and 187, 1945.

induced arterionecrosis resembles somewhat arteriocalcinosis of the Monckeberg type. In man, medionecrosis of the aorta was observed with many diseases, including coronary disease, infectious disease, hypertension, hypotension and endocrine disorders. Ziegler^{3p} and Pearce and Baldauf^{3g} concluded that medionecrosis is the result of ischemia following circulatory disturbances of the vasa vasorum. This mechanism was also emphasized by Erdheim^{1b} and by Schlichter¹¹. The present studies have clearly revealed that this mechanism is operative. In comparing the medionecrotic lesions reproduced in different species by different methods with the lesions in man one concludes that the vascularization of the aorta determines the site, the extent and the severity of these lesions.

The difference in susceptibility to ischemia of the aortic wall in different species is due to the difference in the richness of the vascularization of the aorta. I have found that the rabbit has the poorest vascularization, the dog the best, while that of man is in between. In rabbits vessels over 10 microns are scarce in the aorta. In the media no vessels of this size could be demonstrated. In man the adventitial and medial networks are much less abundant than in the dog. In fact, no extensive medial vasculature of vessels over 10 microns could be demonstrated in the human aorta, nor could such-sized vessels be seen arising directly from the lumen of the aorta. The excellence of the aortic vascularization of the dog may explain why medionecrosis, as well as arteriosclerosis, is so difficult to produce in this species. On the other hand, the poor vascularization of the rabbit's aorta may account for the ease of reproducing medionecrosis and its sequelae (and arteriosclerosis) in this species.

The healing of the medionecrotic lesions and ruptures depends on the adequacy of the collateral circulation. This is sufficiently variable, and so is the primary occlusion, to account for the variability in healing encountered. In the present studies of the vascularity of the dog's aorta I was impressed with the variability of the collateral circulation observed in different animals.

The individual variability of vascularization of the descending aorta is less striking than that of the ascending aorta. This may be an important factor in explaining why necrosis followed by rupture and dissecting aneurysm is more common in the ascending than in the descending aorta. However, medionecrosis of the type found in the ascending aorta does occur in the descending aorta and the arch, as Rottino^{1c} has shown. On routine postmortem examination a large number of such lesions were encountered by him, distributed throughout the aorta. However, it is rarer to find rupture and dissecting aneurysm arising in the arch or the descending aorta, even though these regions have a poorer vascularity than the ascending aorta. It may be that the greater frequency of

rupture and dissection of medionecrosis in the ascending aorta is due, aside from the individual variability of the vascularization of the ascending aorta, to (a) the greater mobility of this region within the pericardial sac, (b) the possibility of impact of the stream of blood leaving the heart and impinging on its walls and (c) the fact that, unlike the descending aorta and the arch, the ascending aorta is not surrounded by connective tissue from the neighboring structures, which has a strengthening action on the walls. Another factor of importance may be the greater frequency of stomas in the intima beyond the ascending aorta. Therefore, compared with the adventitial supply, the relative supply directly from the lumen is even more abundant. This insures a better compensatory system to make up for interruptions in blood supply from the adventitia and prevents total necrosis of the aortic wall.

The role of these peculiarities in the vascularity of different portions of the aorta may also be related to the marked preponderance of atheromatous lesions in the descending aorta.

SUMMARY

The adventitia of the ascending aorta was coagulated in dogs, and the changes in the aortic wall were observed after from six hours to six weeks.

Edema appeared in the inner third of the media and small hemorrhages in its outer third within twelve hours after coagulation.

In the outer and middle thirds of the media, in which the blood supply had been interfered with, diffuse necrosis was observed in the majority of the dogs. In the inner third of the media, which is supplied to a large extent from the lumen of the aorta, only focal necrosis and cyst formation appeared. The process of necrosis was a slow one, involving the muscular constituents before the elastic fibers. It was followed in turn by liquefaction, cyst formation and collagenous fiber replacement. The extent and the speed of necrosis depended on the efficacy of the collateral circulation. Usually the process ended in the third week. There was new formation of blood vessels in the adventitia, and collateral channels developed. Adhesion of the split pericardium also helped in the revascularization.

Proliferation of the intima appeared in the second week, was most extensive in the sixth week and paralleled the medial repair process.

In 1 dog a spontaneous rupture and a dissecting aneurysm of the ascending aorta were produced. In another dog, a hypertensive one, a true small aneurysm appeared.

The incidence of medionecrosis, spontaneous rupture and dissecting aneurysm of the ascending aorta seen in dogs was compared with that seen in man and other species, and with the incidence of such lesions of other parts of the aorta.

COURSE OF WOUND HEALING IN THE SKIN OF MICE UNDER THE INFLUENCE OF CARCINOGENS

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AND

RUTH SILBERBERG, M D

ST LOUIS

PROLONGED application of 3,4-benzpyrene or of 20-methylcholanthrene previous to the making of wounds in the skins of young mice resulted in retardation of the closure of the wounds¹ The delay was due to an inhibition of the migration of the epidermal cells which normally advance into the defects This delay was the more conspicuous as cell proliferation was markedly increased at the margin of the excision and the mitotic cycle of this epithelium failed to return to normal ten days after the wounds had been made, as that of epithelium of untreated skin did

Among the questions arising from these observations were the following (1) Is the cell migration only temporarily suspended, or is it permanently inhibited? (2) Provided complete epithelization does finally occur, would the mitotic counts remain high because of the stimulative effect of the carcinogenic hydrocarbons, or would they drop to normal levels? The answer to the last question seemed of interest also in regard to the problem as to whether a surgical wound may influence the localization of tumors in epidermis treated with such substances We have therefore undertaken a study of the late stages of wound healing in the skins of young mice treated with carcinogenic hydrocarbons

MATERIAL AND METHODS

Sixty-four mice of the inbred strain RFI, 8 weeks old at the beginning of the experiments and kept on a standard diet of Purina dog chow² and water,

This investigation was aided by the David May-Florence G May Fund

From the Snodgras Laboratory of Pathology, City Hospital, and the Pathological Laboratory of the Jewish Hospital

1 Silberberg, M, and Silberberg, R Am J Path **20** 809, 1944, Arch Path **39** 257, 1945

2 The Ralston Purina Company supplies the following list of ingredients of the dog chow meat meal dried skim milk, riboflavine, carotene, cod liver oil,

(Footnote continued on next page)

were used, and litter mate animals of both sexes were as evenly divided among the experimental groups as possible. The two main groups included (1) 32 mice painted with a benzene solution of 20-methylcholanthrene (concentration 0.3 per cent) and (2) 32 mice treated with a benzene solution of 3,4-benzpyrene (concentration 0.3 per cent). These two solutions of carcinogenic substances were applied three times weekly, each application being made with a single stroke of a camel's hair brush, no. 6, on a carefully clipped area of skin of the back. In each group subgroups of 8 animals each were thus treated for two weeks, one month, two months and three months, respectively. At the end of the period of treatment a circular piece of epidermis with some underlying tissue, measuring 4 mm in diameter, was excised from each animal with a pair of curved scissors. Healing of the wounds was allowed to take place, and ten, twenty, thirty or forty-five days after the excisions the animals were killed in pairs between 10 and 11 a. m. The skin containing the scar and adjacent tissue was fixed in solution of formaldehyde U. S. P. diluted 1:10, and serial sections were prepared for histologic study. The further details are the same as those given in previous papers.

HISTOLOGIC EXAMINATION

CONTROLS

As previously shown in untreated control mice, the skin consists of two layers of epidermal cells. In 10,000 basal cells 12 mitoses (a mean) were counted, the ratio between basal and spinous cells being 2:1. The wounds, 4 mm in diameter, made in the skins of these untreated mice were epithelized ten days after excision.

MICE PAINTED WITH 20 METHYLCHOLANTHRENE

Wound Healing After Two Weeks of Application—Marginal Epithelium. Ten days after excision (table 1) the epidermis consisted of four or five rows of epithelial cells, which were larger than those of the untreated controls. Whereas in the latter the number of mitoses had dropped to normal, in the painted animals the mean mitotic count was two and one-fourth times higher. After twenty or thirty days there were still three to five cell rows and two and one-fourth or two and three-fourths as many mitoses as ordinarily. Forty-five days after operation the mean mitotic count had fallen to one and one-fourth times the normal, this coincided with a decrease in the number of rows of epithelial cells to three or two. The details are given in table 1.

New Epithelium. Ten or twenty days after the making of the wounds the defects were closed and covered by from five to eight rows of large epithelial cells. The number of mitoses was normal or slightly above normal with the exception of animal 350, in which the epithelium was exceedingly thick (seven

brewers' dried yeast, wheat germ, wheat cereal, corn grits, corn cereal, dried beet pulp, molasses, steamed bone meal and iodized salt. The chemical analysis is

| | Crude, % | Digestible, % |
|-----------------------|----------|---------------|
| Protein | 23.0 | 19.0 |
| Fat | 5.0 | 4.7 |
| Fiber | 4.0 | |
| Ash | 7.0 | |
| Nitrogen-free extract | 54.0 | 48.0 |
| Moisture | 7.0 | |

or eight rows of cells) and the number of mitoses four times the normal. After thirty or forty-five days the epidermis was composed of two or three rows of cells, thus approaching the usual condition, while the mean mitotic counts had dropped to one and a half or one-third the usual values. Even then, however, the scar could be recognized in the section by the presence of more fibrous tissue in the subcutis and by the absence of appendages.

Wound Healing After One Month of Application—Marginal Epithelium Ten days after excision the epidermis was thickened (four to eight rows of cells), and in the enlarged cells the mean count of mitoses was five and a half times the normal. The epithelium was covered by a layer of keratin. From twenty days after the operation on, the epithelium became much thinner (two to four rows of cells), and the number of mitoses likewise showed a return to normal.

New Epithelium In animal 335 the wound was about to close, whereas in mouse 336 the defect was completely epithelized. In the former the epidermal layer was thicker (nine rows of cells) and more mitoses (nine times the normal) were found than in the latter, which showed six or seven rows of cells and five times the normal number of mitoses. After twenty or thirty days there were only four or five rows of epithelial cells and the means of mitotic counts were lower than before (two and one-fourth and one and three-fourths the normal, respectively). After forty-five days the epidermis consisted of two or three rows of small epithelial cells, with a mean mitotic count of half the usual values only.

Wound Healing After Two Months of Application—Marginal Epithelium Ten or twenty days after the making of the wound there were four or six rows of epithelial cells covered by a thick layer of keratin, and the mean mitotic counts were five and a half or six and one-fourth times as high as ordinary. After thirty or forty-five days, however, the keratinization was less accentuated, the epidermis was composed of only three or four rows of epithelial cells, and the numbers of mitoses had declined to a mean of three, or one and three-fourths times the normal.

New Epithelium After ten days the defects were still open in both animals. The epithelium migrating into the wound contained six to eight cell rows, and the mean mitotic count was three and three-fourths times the normal. Twelve to fourteen days after excision a marked narrowing of the wounds had been noted in the living animals, after sixteen days the crusts covering the defects were sloughed off and scars became visible. Histologically, after twenty days the number of epithelial cell rows had decreased to four or five, and the numbers of mitoses had dropped to a mean of one and three-fourths times the normal. Subsequently there was a further thinning of the epithelium with a further decrease in the numbers of mitoses, the mean of which was three-fourths the normal forty-five days after operation. In 3 of the animals observed for thirty or forty-five days after excision papillomas had developed at some distance from the scars. The healing of the wounds was apparently not influenced by the presence of these tumors.

Wound Healing After Three Months of Application—Marginal Epithelium Ten days after the wounds had been made the epithelium consisted of six to ten rows of epithelial cells, above which there was a thick layer of keratin. The mean mitotic count of the basal cells was as high as ten times the normal. At twenty days and later the number of the epithelial cell rows decreased somewhat, to from five to eight layers, there was less keratinization, and the numbers of mitoses dropped likewise. The mean mitotic counts were from eight and a half

TABLE 1—*Methylcholanthrene-Treated Animals*

| Duration of Painting | Duration of Healing | Animal | Rows of Cells in | | Mean Number of Mitoses with Their Maximum and Minimum Deviations in Multiples of the Normal | | | | Type of Tumor |
|----------------------|---------------------|--------|------------------|-----------|---|---------|--------------------|--|---------------|
| | | | Old Epith | New Epith | Old Epith | | New Epith | | |
| 2 weeks | 10 days | 348 | 4 | 6 | 2½ max 3½ min 1½ | | 1 max 1 min 1 | | |
| | | 349 | 5 | 6 | 2 max 2 min 2 | | 1½ max 2 min 1 | | |
| | | | | | Mean 2¼ | | Mean 1¼ | | |
| | 20 days | 350 | 4-5 | 7-8 | 2½ max 3 min 2 | | 4 max 4½ min 3 | | |
| | | 351 | 3-4 | 5-6 | 2 max 3 min 2 | | 1½ max 2 min 1 | | |
| | | | | | Mean 2¼ | | Mean 2¾ | | |
| | 30 days | 352 | 3 | 3 | 1½ max 2 min 1 | | ½ max 1 min ½ | | |
| | | 353 | 4 | 3 | 4 max 5 min 3 | | 2½ max 3 min 2 | | |
| | | | | Mean 2¾ | | Mean 1½ | | | |
| | 45 days | 354 | 2-3 | 2-3 | 1½ max 2 min 1 | | ¾ max ¾ min 0 | | |
| | | 355 | 2-3 | 3 | 1 max 1 min 1 | | ½ max ½ min 0 | | |
| | | | | | Mean 1¼ | | Mean ½ | | |
| 1 month | 10 days | 335 | 8 | 9 | 6 max 9 min 5 | | 9 max 10 min 7 | | |
| | | 336 | 4-5 | 6-7 | 5 max 6 min 3½ | | 5 max 6 min 4½ | | |
| | | | | | Mean 5½ | | Mean 7 | | |
| | 20 days | 337 | 2-3 | 4-7 | 1 max 1 min 1 | | 3 max 4½ min 2½ | | |
| | | 362 | 3 | 4 | 1 max 1 min 1 | | 1½ max 2 min 1 | | |
| | | | | Mean 1 | | Mean 2¼ | | | |
| | 30 days | 356 | 3 | 4 | 1 max 1 min 1 | | 2 max 2½ min 1 | | |
| | | 357 | 4 | 5 | 2 max 3 min 1 | | 1½ max 2½ min 1 | | |
| | | | | Mean 1½ | | Mean 1¾ | | | |
| | 45 days | 358 | 4 | 3 | 1 max 1 min 1 | | ½ max ¾ min ½ | | |
| | | 359 | 2 | 2-3 | 1 max 1½ min 1 | | ¾ max ¾ min ½ | | |
| | | | | | Mean 1 | | Mean ½ | | |
| 2 months | 10 days | 363 | 4 | 6 | 4½ max 5 min 3 | | 3 max 4 min 2½ | | |
| | | 364 | 6 | 8 | 6½ max 7½ min 5½ | | 4½ max 6 min 4 | | |
| | | | | | Mean 5½ | | Mean 3¾ | | |
| | 20 days | 365 | 6 | 4-5 | 6 max 7 min 5 | | 1½ max 2 min 1 | | |
| | | 366 | 5 | 4 | 6½ max 8 min 6 | | 2 max 2½ min 1½ | | |
| | | | | Mean 6¼ | | Mean 1¾ | | | |
| | 30 days | 367 | 3 | 6 | 3 max 4 min 2½ | | 2 max 2½ min 1 | | Papillomas |
| | | 368 | 3-4 | 2-3 | 3 max 4 min 2 | | 2 max 2½ min 1½ | | |
| | | | | Mean 3 | | Mean 2 | | | |
| | 45 days | 369 | 3-4 | 3 | 1½ max 2 min 1 | | ½ max 1 min ½ | | Papilloma |
| | | 370 | 4 | 3-4 | 2 max 2½ min 1½ | | 1 max 1½ min ½ | | Papilloma |
| | | | | | Mean 1¾ | | Mean ¾ | | |

| Duration of Painting | Duration of Healing | Animal | Rows of Cells in | | Mean Number of Mitoses with Their Maximum and Minimum Deviations in Multiples of the Normal | | | | Type of Tumor |
|----------------------|---------------------|--------|------------------|-----------|---|-----------------------------|-----------|------------------------------|---------------|
| | | | Old Epith | New Epith | Old Epith | | New Epith | | |
| | | | | | max | min | max | min | |
| 3 months | 10 days | 338 | 6 | 10 | 6 | max 8 min 4½ | 7½ | max 8½ min 6½ | Papillomas |
| | | 339 | 10 | 12 | 14 | max 16 min 12 Mean 10 | 17 | max 20 min 14 Mean 12¾ | Papilloma |
| | | 340 | 5 | 7 | 9½ | max 15 min 6 | 4 | max 4½ min 3½ | Papillomas |
| | | 341 | 7 | 5 | 7½ | max 8 min 7 Mean 8½ | 4 | max 5 min 3 Mean 4 | Papillomas |
| | | 342 | 7 | 9 | 5 | max 6 min 4 | 3 | max 4 min 3½ | Papillomas |
| | | 343 | 6 | 8-9 | 6½ | max 8 min 4½ Mean 5¾ | 2½ | max 3 min 2 Mean 2¾ | Papillomas |
| | 45 days | 344 | 8 | 10 | 4 | max 5½ min 4 | 2½ | max 3 min 1½ | Papillomas |
| | | 345 | 6 | 4 | 4 | max 5 min 2½ Mean 4 | 5 | max 6 min 4 Mean 3¾ | Papillomas |

to four times higher than usual and showed wide variations. This was apparently due to the presence of tumors, which had developed near the wounds as well as at some distance. The closer the neoplasms to the regenerating epithelium, the more numerous were the mitoses in the marginal epithelium, the farther away the new growths were, the fewer were the mitoses at the edges of the excisions. No tumor was noted in the wounds or in the scars themselves.

New Epithelium. The wounds were still open after ten days. However, they narrowed markedly at the end of the second week, and they were healed after twenty days. Histologically, ten days after operation the epithelium contained from ten to twelve rows of cells, and the mean mitotic count was twelve and one-fourth times higher than usual. Subsequently, the epithelial layer in the scars decreased in size, but close to the tumors the epithelium remained markedly thickened. The numbers of mitoses showed likewise a decline, and after forty-five days a mean of three and three-fourths times the normal was found. Again the mitoses were the more numerous the closer the scars were to the tumors. In some mice multiple papilloma had developed, which accounted for wide variations in the numbers of mitoses, particularly if the new growth approached the regenerated area, but in no case did a tumor originate in the area of excision.

MICE PAINTED WITH 3,4 BENZOPYRENE

Wound Healing After Two Weeks' Application.—Marginal Epithelium. Ten days after operation the epidermis showed a surface layer of keratin and contained three or four layers of epithelial cells (table 2). The mean mitotic count of the basal cells was four and three-fourths times the normal. During the late stages the epithelium decreased slightly in thickness (two to four rows of cells) and the mean of the mitotic counts ranged between two and one-half and one and three-fourths times the usual figures. The details are presented in table 2.

New Epithelium. Ten days after excision the wounds were epithelized and covered by four to eight rows of large epithelial cells. The mean mitotic count

TABLE 2—*Benzpyrene-Treated Animals*

| Duration of Painting | Duration of Healing | Animal | Rows of Cells in | | Mean Number of Mitoses with Their Maximum and Minimum Deviations in Multiples of the Normal | | | | Type of Tumor |
|----------------------------|---------------------------|--------|---------------------|--------------|--|----------|---------------|-----------|----------------------------|
| | | | Old Epith | New Epith | Old Epith | | New Epith | | |
| 2 weeks | 10 days | 373 | 4 | 7 8 | 5½ max min | 6½ 4½ | 10 max min | 12 8 | |
| | | 374 | 3 | 4 5 | 4 max min | 4½ 3½ | 3½ max min | 4½ 2½ | |
| | | | | | Mean 4¾ | | Mean 6¾ | | |
| | 20 days | 375 | 3 | 4 5 | 3 max min | 4 2½ | 1½ max min | 2 1 | |
| | | 376 | 4 | 4 | 2 max min | 3 2 | 1 max min | 1½ ½ | |
| | | | | | Mean 2½ | | Mean 1¾ | | |
| | 30 days | 377 | 4 | 3 | 1½ max min | 2 1 | ½ max min | ¾ 0 | |
| | | 378 | 3 4 | 3 | 2 max min | 2 2 | ¾ max min | ¾ ½ | |
| | | | | | Mean 1¾ | | Mean ¾ | | |
| | 45 days | 379 | 3 | 4 | 3½ max min | 4½ 2½ | ½ max min | ¾ 0 | |
| | | 380 | 2 3 | 3 | 1 max min | 1 1 | ½ max min | ½ 0 | |
| | | | | | Mean 2¼ | | Mean ½ | | |
| 1 month | 10 days | 383 | 4 | 5-6 | 3 max min | 4 2½ | 6 max min | 7½ 5 | |
| | | 384 | 5-6 | 8 | 6 max min | 7 5 | 11 max min | 13 10 | |
| | | | | | Mean 4½ | | Mean 8½ | | |
| | 20 days | 385 | 4 | 4 5 | 5½ max min | 6 4 | 2½ max min | 3 2 | |
| | | 386 | 3-4 | 4 5 | 2½ max min | 2½ 2 | 2½ max min | 3 2 | |
| | | | | | Mean 4 | | Mean 2½ | | |
| | 30 days | 387 | 3 4 | 5 | 1½ max min | 2 1 | ¾ max min | 1 ½ | |
| | | 388 | 3 | 2 | 1 max min | 1½ 1 | ¾ max min | 1 ¾ | |
| | | | | | Mean 1¾ | | Mean ¾ | | |
| | 45 days | 389 | 4 | 3 4 | 2 max min | 2½ 2 | ¾ max min | ¾ 0 | |
| | | 390 | 4 | 3 | 1 max min | 1 ¾ | ¾ max min | ¾ 0 | |
| | | | | | Mean 1½ | | Mean ½ | | |
| 2 months | 10 days | 392b | 6 | 7 8 | 7½ max min | 9 6 | 11 max min | 12 10 | Carcinoma |
| | | 393 | 6-7 | 9 10 | 7 max min | 8 6 | 11 max min | 12½ 10 | |
| | | | | | Mean 7¼ | | Mean 11 | | |
| | 20 days | 394 | 4 | 3 | 4½ max min | 6 4 | 1½ max min | 2 1 | |
| | | 395 | 4 5 | 4 | 8 max min | 9 7 | 2½ max min | 3 2 | |
| | | | | | Mean 6¾ | | Mean 2 | | |
| | 30 days | 396 | 6-7 | 4 | 11 max min | 12 10 | 11 max min | 12 10 | Carcinoma |
| | | 397 | 4 | 9 10 | 10 max min | 11 9 | 15 max min | 17 14 | Carcinoma and papilloma |
| | | | | | Mean 10½ | | Mean 13 | | |
| | 45 days | 398 | 6 | 5-6 | 2 max min | 2 1½ | 1 max min | 2 ¾ | Carcinoma |
| | | 399 | 4 5 | 3 4 | 3 max min | 4½ 2½ | 2 max min | 2½ 1½ | Carcinoma |
| | | | | | Mean 2½ | | Mean 1½ | | |

| Duration of Painting | Duration of Healing | Animal | Rows of Cells in | | Mean Number of Mitoses with Their Maximum and Minimum Deviations in Multiples of the Normal | | | | | | Type of Tumor |
|----------------------------|---------------------------|--------|---------------------|--------------|--|----------|--------|-----------|---------|---------|-----------------------------|
| | | | Old Epith | New Epith | Old Epith | | | New Epith | | | |
| | | | | | max | min | Mean | max | min | Mean | |
| 3 months | 10 days | 402 | 5 6 | 7 8 | 10 | max 11 | min 10 | 14 | max 15 | min 13 | Papillocarci- noma |
| | | 403 | 7 | 5 | 8 | max 9½ | min 7½ | 12 | max 13 | min 11½ | Carcinoma |
| | | | | | | Mean 9 | | | Mean 13 | | |
| | 20 days | 404 | 6 | 4 5 | 7½ | max 9 | min 6 | 10 | max 11 | min 9½ | Carcinoma |
| | | 405 | 6 | 8 | 8 | max 10 | min 7 | 9½ | max 11 | min 7½ | Carcinoma |
| | | | | | | Mean 7¾ | | | Mean 9¾ | | |
| | 30 days | 406 | 5 | 6 7 | 12½ | max 14 | min 11 | 14 | max 15 | min 13 | Carcinoma and papillomas |
| | | 407 | 6 7 | 8 | 10 | max 10½ | min 10 | 10 | max 11 | min 9 | Carcinoma and papilloma |
| | | | | | | Mean 11¾ | | | Mean 12 | | |
| | 45 days | 408 | 7 | 8 9 | 14 | max 15 | min 12 | 14 | max 15 | min 13 | Carcinoma and papillomas |
| | | 409 | 6 | 6 | 12 | max 14 | min 10 | 12 | max 14 | min 10 | Carcinoma and papillomas |
| | | | | | | Mean 13 | | | Mean 13 | | |

was six and three-fourths times as high as ordinarily. After twenty days there were four or five rows of cells and a decline in the number of mitoses to one and one-fourth the normal. After thirty or forty-five days the scar showed three or four rows of small epithelial cells and a mitotic count of one-half or one-fourth the normal.

Wound Healing After One Month of Application—Marginal Epithelium. Ten days after operation the epidermis contained four to six rows of epithelial cells, covered by a layer of keratin. Even after forty-five days of observation the epidermis remained thickened (three or four rows of cells). After twenty days the mean number of mitoses was still four times the normal. After thirty or forty-five days there was a steep drop in the mitotic counts to about normal.

New Epithelium. After ten days the defects were not yet completely covered with epithelium. The epithelial-tongues advancing from the margins of the wound were composed of from five to eight rows of cells and the mean number of mitoses therein was eight and a half times the normal. Grossly, the defects were healed between fourteen and sixteen days after excision. After twenty days, coincident with a decline in the number of rows of epithelial cells to four or five, two and a half times (mean) as many mitoses were found as ordinarily. At later stages the epithelium became still thinner (three or four rows of cells), and the mean mitotic count dropped to below normal.

Wound Healing After Two Months of Application—Marginal Epithelium. Carcinoma was present in 5 of the 8 mice. In 2 of the mice it was in such close approximation to the scar that the effect of healing on the mitotic count could not be determined. In the other 3 mice it was sufficiently distant from the line of excision to allow a study of the margin of the wound. Observations made in these 3 mice and on the remaining 3 mice, in which no tumors were present, provided the basis for the following description. Ten days after operation the epidermis was covered by a thick layer of keratin and consisted of six or seven

rows of epithelial cells with a mean mitotic count of seven and one-fourth times the normal. After twenty days the number of cell rows had decreased to four or five and that of the mitoses to a mean of six and one-fourth times the usual values. After forty-five days the mean mitotic count was down to two and one-half times the normal.

New Epithelium Ten days subsequent to excision the defects were not yet epithelized. The epithelial tongues were thick and contained from seven to ten rows of cells showing a mean mitotic count of eleven times the normal. Subsequently, the wounds closed, but the numbers of mitoses showed wide variations. After twenty days and later the mean number of mitoses was slightly higher than ordinarily, the individual values ranging between normal and two and a half times the normal.

Wound Healing After Three Months' Application—**Marginal Epithelium** Only in the animals observed for ten days were the tumors sufficiently distant from the wounds so that cell counts could be made. All animals had carcinoma in the painted skin. Some had in addition multiple papilloma. Ten days after operation the epidermis consisted of five to seven layers of epithelial cells and a thick layer of keratin. The mean mitotic count was nine times as high as usual. Subsequently the number of mitoses remained high or even increased, particularly near the neoplasms.

New Epithelium The wounds were not epithelized ten days after operation. The epithelial tongues consisted of five to eight rows of cells, the mean mitotic count being thirteen times the normal. Since at later stages tumors had grown over the scars, the high mitotic counts obtained cannot be considered as representing the true figures for mitoses in the scarred epithelium.

COMMENT

Wounds 4 mm in diameter made in untreated skin of young mice were closed ten days after excision. As reported previously,¹ when methylcholanthrene or benzpyrene was applied to the epidermis for two weeks or for one month previous to the making of the defect, this treatment caused a slight acceleration of healing comparable to that seen after painting with the solvent benzene alone. If the carcinogens were applied to the epidermis as long as two or three months previous to the operation, healing of the excised area was completed after about fifteen days, that is, five days later than in nontreated epidermis. Carcinoma was present in every animal to the skin of which benzpyrene was applied for three months and in 5 of 8 mice painted for two months. Papilloma was noted in 7 of 8 animals treated with methylcholanthrene for three months and in 3 of 8 mice painted for two months and observed thereafter for thirty or forty-five days. No carcinoma was obtained in the methylcholanthrene-treated animals.

Wounds made in untreated skin showed a peak of mitotic activity in both the old marginal and the new regenerated epithelium five days after excision. After ten days the number of mitoses was normal.

again. However, in the epidermis treated with carcinogens the number of mitoses remained high even after ten days of repair. In the present series the periods of observation were sufficiently long to allow complete epithelization of the defects.

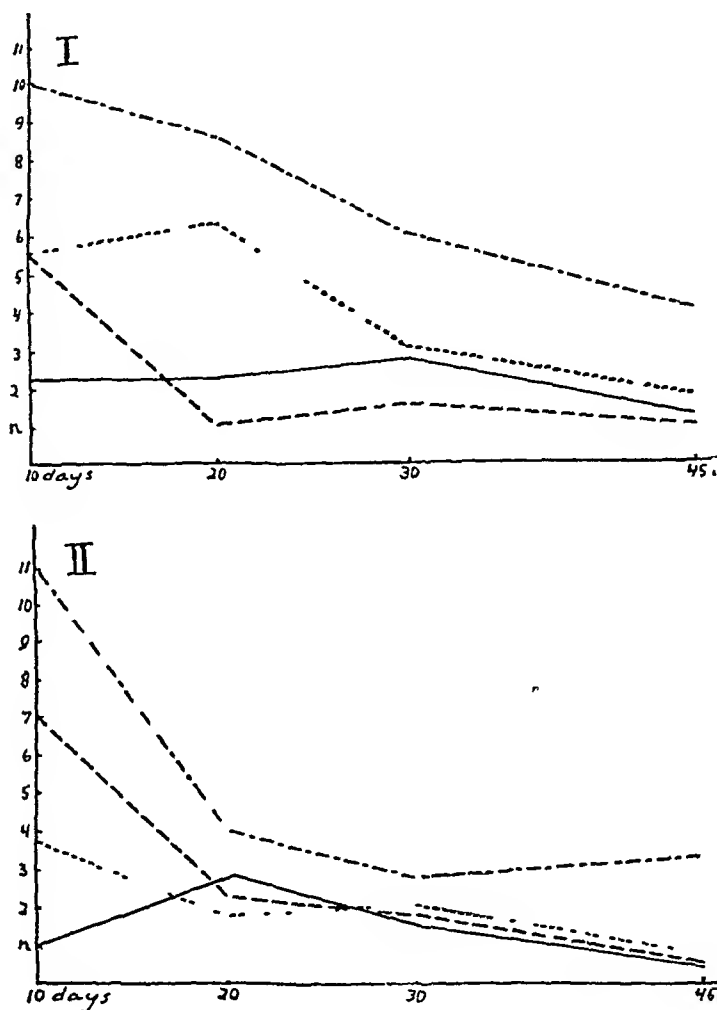


Chart 1—Mitotic cycle in the old epithelium from the tenth to the forty-fifth day of wound repair in mice treated with 20-methylcholanthrene for two weeks (straight line), one month (broken line), two months (dotted line) or three months (dot and dash line), *n* stands for the normal value and the numbers above *n* for multiples of the normal.

Chart 2—Mitotic cycle in the new regenerating epithelium from the tenth to the forty-fifth day of wound repair in mice treated with 20-methylcholanthrene for two weeks (straight line), one month (broken line), two months (dotted line) or three months (dot and dash line), *n* stands for the normal value and the numbers above *n* for multiples of the normal.

In charts 1 to 4 the mitotic counts made in the marginal and in the regenerated epithelium during later stages of wound healing are presented. Since in untreated animals the number of mitoses had

returned to normal after ten days, this period was chosen as the starting point of the present investigation and normal mice are not included in these charts. Excluded from the charts are, furthermore, those mice in which carcinoma had invaded the scars or was so close to them that mitotic counts of nontumorous epithelium could not be made near the regenerating epidermis. Among these animals were

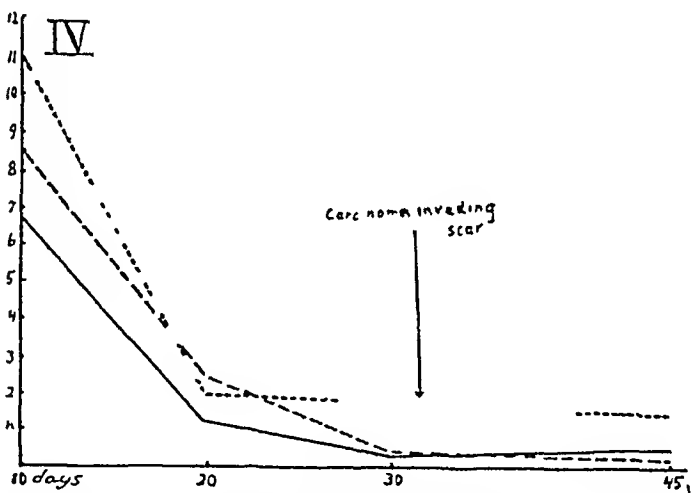
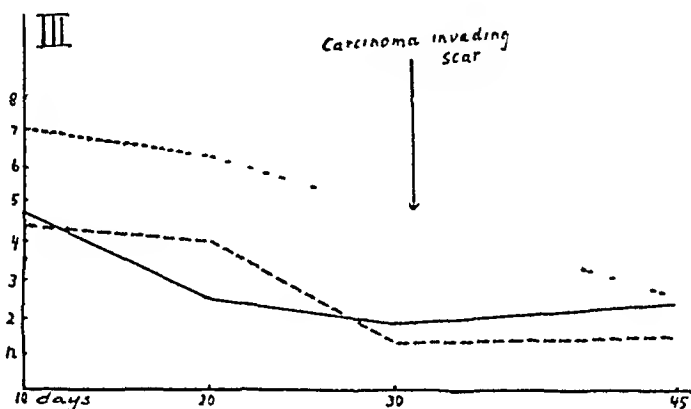


Chart 3—Mitotic cycle in the old epithelium from the tenth to the forty-fifth day of wound repair in mice treated with 3,4-benzpyrene for two weeks (straight line), one month (broken line) or two months (dotted line), "n" stands for the normal values and the numbers above "n" for multiples of the normal

Chart 4—Mitotic cycle in the new regenerating epithelium from the tenth to the forty-fifth day of wound repair in mice treated with 3,4-benzpyrene for two weeks (straight line), one month (broken line) or two months (dotted line), "n" stands for the normal values and the numbers above "n" for multiples of the normal

all those painted with benzpyrene for three months and 2 of 8 painted for two months. Mitotic counts could be obtained in and around the wounds in all mice painted with methylcholanthrene, without interference from the papillomatous growths. After periods of treat-

ment with either carcinogen ranging up to two months the mitotic counts of the marginal and the regenerated epithelium showed a tendency to return to normal values. The slight rises seen here and there are probably due to some irregularities beyond control either in the wound itself or in its location or in the local rhythmic variations of the mitoses in the epidermis. Thirty days subsequent to excision the mitotic counts dropped to levels ranging from three times the normal to below normal. Slowest in returning to lower values were, according to expectation, the mitotic counts following three months of painting with methylcholanthrene. They did not decline below four times the ordinary number. However, this constituted a considerable fall from their maximum of ten times the usual figures, seen after ten days of repair, and it corresponded in absolute values to the drop of from five and a half times the normal to normal after one month of painting. On the whole, conditions in the regenerated epithelium paralleled those in the marginal epithelium, although the mitotic activity declined more rapidly in the former. A sudden fall usually occurred between the tenth and the twentieth day after operation. After forty-five days, in most instances, the number of mitoses dropped even to below normal, thus indicating a particularly inactive epithelium. Only after methylcholanthrene had been applied three months or benzpyrene for two months were the counts above normal. This may well present merely a delayed return to normal.

The results of the present experiments confirm our previous observations concerning the effects of carcinogens on the course of repair in the skin of growing mice. The return of the mitotic counts to normal values was again delayed, a decline of the mitotic activity in both old and new epithelium was noted only after the defects were epithelized, that is, between the tenth and the twentieth day of healing. Thus, in accordance with the observations of Loeb,³ the meeting of the epithelial tongues in the centers of the wounds resulted in an effective inhibition of the proliferation of the regenerating epithelium. This is thus true not only of the normal skin but also of the epidermis painted with carcinogens. What made the migrating epithelial cells overcome the forces that had inhibited their movement at earlier stages of wound healing is uncertain. Since the treatment with the carcinogen had been discontinued at the time of the making of the wound, the effect of each carcinogenic agent lessened progressively as the interval between excision and the date of killing increased. It is thus possible that the stimulus of the wounds after a while became

³ Loeb, L. Arch f Entwcklingsmechan d Organ 6 297, 1898. Loeb, L., and Spain, K. C. J Exper Med 23 107, 1916.

predominant over the effect of the carcinogen. Moreover, the regenerating epithelium was never exposed to the direct influence of the carcinogen, and this might be another reason that it behaved in a manner similar to the untreated epithelium. Experiments with cutaneous transplants are in progress which will further test these phenomena and in particular the possible role of the base of the wound in the migration of the epithelium.

With mitoses in the new epithelium at a nearly normal level, it is not surprising that tumors did not develop in the scars and that we did not find any correlation between wound healing and tumor formation. Single or multiple neoplasms developed in various places within or around the painted areas, near or at a distance from the line of excision. On the other hand, carcinomas originating at some distance from the wound often invaded and completely obliterated the regeneration areas secondarily. Failure of new growths to originate in healed wounds of mice has been reported also by MacKenzie and Rous⁴ and Brunschwig, Tschetter and Pissell,⁵ although the former investigators noticed tumor formation under similar conditions in the ears of rabbits. According to Mottram,⁶ scarring did not influence the number of tumors in the skin of methylcholanthrene-treated mice, however, the appearance of these tumors was hastened. Pullinger⁷ found the risk of a tumor developing in skin treated with benzpyrene increased by 13 per cent after a single surgical excision. Recently Lacassagne and Latarjet,⁸ likewise studying the development of carcinoma in healed wounds, observed a retardation of the downgrowth of regenerating epidermal appendages in the dermis. These authors attributed the failure of tumors to develop in the scars to the absence therein of appendages, which usually show considerable mitotic activity. On the other hand, they correlated the appearance of tumors at the edges of the scars to the presence of hyperplastic hair follicles and sebaceous glands. However, there is at the margin of the defects a marked increase of mitotic activity of the epithelium irrespective of the appendages. This mitotic stimulation might, under certain conditions, play a part in localizing a tumor at the edge of a healed wound or cause a neoplasm to appear at an earlier date than it would in intact skin. But in our experiments there was no

4 MacKenzie, I, and Rous, P. *J. Exper. Med.* **73** 391, 1941.

5 Brunschwig, A., Tschetter, D., and Pissell, A. D. *Ann. Surg.* **106** 1084, 1937.

6 Mottram, J. C. *J. Path. & Bact.* **56** 391, 1944.

7 Pullinger, B. D. *J. Path. & Bact.* **57** 467, 1945.

8 Lacassagne, A., and Latarjet, R. *Cancer Research* **6** 183, 1946.

such stimulating effect, there was, on the contrary, a definite trend of the epithelium toward a return to a resting state

SUMMARY

The inhibition of the epithelization of wounds made in the skins of young mice treated with 3, 4-benzpyrene or 20-methylcholanthrene for two or three months previous to the making of the defects is temporary. The regenerating epithelium overcomes the forces opposing its migration as it advances into the wound at the end of the second or the beginning of the third week of healing. Coinciding with the epithelization of the defect is a fall in the mitotic activity in both the old marginal and the new regenerated epithelium. This tendency of the epithelium to return to a resting state is considered the reason that, under the present experimental conditions, no correlation was noted between the former site of the wound and the place of tumor formation.

DISSEMINATED RETICULOENDOTHELIAL TUMOR OF THE BONE MARROW WITH NODULAR OSTEOSCLEROSIS

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IN THE CASE to be described a condition was presented which is closely related to reticuloendotheliosis, or aleukemic reticuloendotheliosis, or systemic hyperplasia of the reticuloendothelial cells, but which is different in some respects. It is not a diffuse disorder, whereas the others are. It is limited to a number of circumscribed areas of the bone marrow and is associated with marked bone formation that is likewise limited to the affected areas. The fact that the disease process is not generalized but disseminated and multiple and that the lesion is a true neoplasm of reticuloendothelial cells of the marrow would warrant its classification as multiple reticuloendothelial myeloma, however, the name "myeloma" denominates a well characterized disease associated not only with the proliferation of cells of the marrow but also with profound disturbances of the protein metabolism. These were not displayed in the present observation.

REPORT OF A CASE

A 60 year old white American woman was admitted to Stanford University Hospital for observation of hypertensive disease (blood pressure 200 systolic and 120 diastolic) of about a year's duration, during which time she had lost some weight. The heart was slightly enlarged. The liver and the spleen were of normal size. No lymph nodes were palpated. No masses or other signs of tumor were detected.

Röntgen Examination—Excretory urography carried out in an attempt to determine the cause of hypertension revealed no abnormalities of the urinary tract. Plain roentgenograms disclosed numerous rounded sclerotic densities of various sizes in the innominate bones, the sacrum, the lumbar vertebrae, the ribs and the calvarium. The fifth lumbar vertebra was entirely dense.

The nature of these densities was not apparent. The possibility of bone-forming tumor metastases or of Hodgkin's disease with atypical skeletal distribution was considered.

Laboratory Examination—The red blood cell count was 4,360,000, the hemoglobin content, 78 per cent (Sahli), the color index, 90. The leukocyte count was 14,000. The total neutrophil percentage was 80, 20 per cent were banded and 60 per cent segmented. Eosinophils were 4 per cent, basophils, 1 per cent, lymphocytes, 11 per cent, macrocytes, 4 per cent. The platelet count was 401,120. Of the erythrocytes, 07 per cent were reticulocytes. The hematocrit reading was 36.5.

From the Department of Radiology, Stanford University School of Medicine



Fig 1—Dorsal view of the sacroiliac area. Note the outstanding density of the body of the fifth lumbar vertebra and of adjacent parts of the sacral bone. Numerous rounded densities may be seen in the sacral wings and in the innominate and pubic bones.

Fig 2—Left lateral view of the lumbar portion of the spine. The bodies of the second and fourth lumbar vertebrae show several rounded densities. The body of the fifth lumbar vertebra is dense in its whole extent.

Fig 3—Anterior view of the chest. The anterior portions of the second and third ribs on the right and the third rib on the left reveal a dense area. Bone was taken from the distal end of the second rib on the right for biopsy.

per cent The red cells varied in size and color but not in shape The leukocytes showed no abnormality The platelets were numerous and apparently normal from the standpoint of morphology Marrow obtained by sternal puncture showed an increased number of plasma cells of the normal (Marschalko) type and an increased number of megakaryocytes No neoplastic cells were present

Further tests revealed high serum chlorides (683 mg per hundred cubic centimeters), high serum inorganic phosphates (49 mg per hundred cubic centimeters) and a high basal metabolic rate (+35 to 37) All other values were within normal limits No Bence Jones protein was detected Serum albumin amounted to 3.8 Gm and globulin to 2.7 Gm per hundred cubic centimeters The albumin-globulin ratio was 1.407 (normal), the serum protein was 6.87 mg per hundred cubic centimeters

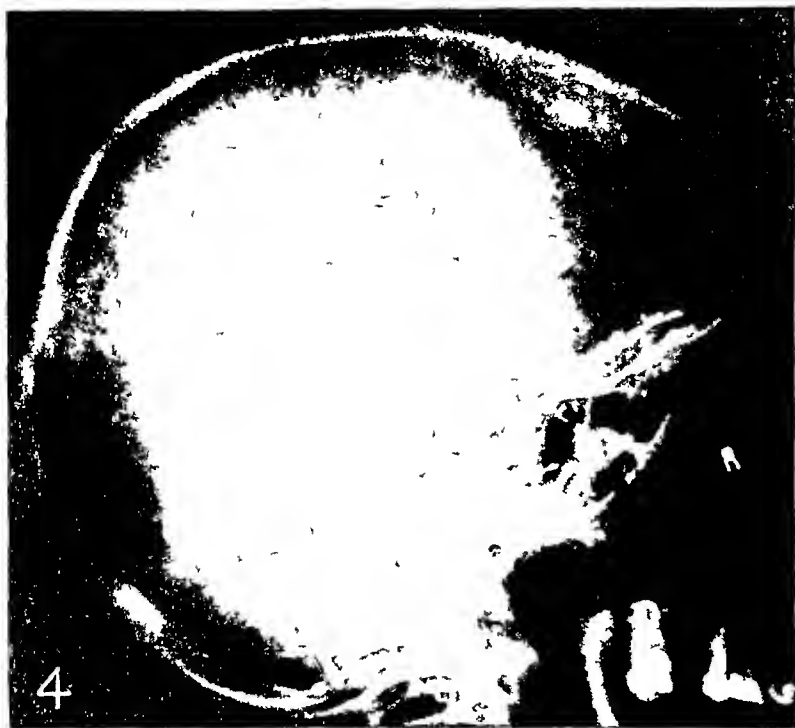


Fig 4—Irregular densities, some minute and some larger, are seen in the bones of the cranial vault The parietal bones are mainly affected

Fig 5—Roentgenogram of the biopsy specimen taken from the distal end of the second rib on the right, showing a circumscribed area of bone density



Since the results of laboratory tests, physical examination and clinical observation failed to divulge any sign of a primary tumor, and since no explanation for the striking roentgenologic findings could be given, biopsy of bone was done and reported on as follows (Dr William H Carnes, of the department of pathology of Stanford University School of Medicine) "The interstices between the bone trabeculae were filled with a fine but distinct collagenous stroma and abundant reticulum The cells varied somewhat in size as well as shape The nuclei were medium sized and often eccentric and were composed of a dark-staining coarse chromatin, which was frequently distributed in a peripheral or a radial pattern, with identifiable nucleoli No mitoses were seen The cytoplasm was abundant, with relatively basophilic staining and frequently a distinct peri-



Fig 6 (case 1)—Photomicrograph ($\times 189$) showing tumor cells of irregular shapes and sizes, with abundant cytoplasm and dark-staining small nuclei. Note the large amount of intercellular stroma. There is lacunar erosion of a bone trabeculum at the lower end of the picture. Hematoxylin-eosin.

Fig 7—Photomicrograph ($\times 1415$) showing massive new formation of bone tissue with dense lamellated bone structures replacing the cortex (at the left and the middle of the picture). Newly formed trabeculae of irregular distribution (almost mosaic-shaped lamellas) may be seen at the left. Note the tumor tissue in haversian canaliculae and in marrow spaces. Hematoxylin-eosin.

nuclear clear zone. Occasionally, the cytoplasm contained a few fine vacuoles, especially at the cell margin. A few characteristic large round cytoplasmic inclusions were seen, indistinguishable from Russell's fuchsin bodies. This cellular proliferation corresponded closely to the bone hyperplasia in all sections and extended only a short distance beyond it. Outside the lesions, the bony trabeculae, the cortex and the marrow were normal. The neoplastic nature of the lesion was agreed on by all who examined the slides; however, the cells were not typical myeloma cells, and the abundance of stroma, reticulum and new bone formation was striking."

In view of the microscopic appearance of the tumor, showing a large amount of reticulum and collagenous stroma, and the resemblance of its cellular elements to endothelium, with signs of phagocytosis and vacuolation, it was stated to be reticuloendothelial in origin.

The patient was discharged when diagnostic procedures had been completed. She was readmitted two months later with signs of cardiac decompensation. The liver was now somewhat enlarged (congested). Repeated and additional laboratory studies provided no further information. Hepatolienography with intravenous injection of a colloidal suspension of thorium dioxide showed normal and homogeneous phagocytosis of the thorium dioxide on the part of the reticuloendothelium of the liver and the spleen.

After discharge the patient's condition steadily declined, and she died with signs of congestive failure eight months after her first admission. Permission for postmortem examination was refused.

COMMENT

Histologic examination proved that we were dealing with a disseminated neoplastic lesion of the bone marrow of reticuloendothelial origin. Whether or not other organs rich in reticuloendothelium participated in the disease process was impossible to determine without autopsy, however, none of the clinical or laboratory findings indicated the presence of lesions in internal organs. The only evidence of foci of disease existed in the marrow of spongy bones, ribs, the calvarium, the spinal column and the pelvis. These are the bones in which multiple myeloma commonly occurs.

Roentgenologically, the distinguishing feature of this case was the conspicuous bone formation within areas occupied by the tumor. Microscopic examination showed that this bone tissue was formed in various ways. Dense lamellated bone was deposited on the surface of the cortex by reason of periosteal apposition of bone tissue in the tumor area. In many places this newly formed bone replaced the entire cortex, indicating that previous destruction was followed by regeneration. Irregular, massive, nonlamellated bone trabeculae intruded at identical locations into the marrow cavity. In other areas it was evident that reticular and fibrous interstices of the tumor were submerged into the newly formed nonlamellated bone spicules, indicating that these originated as bony metaplasia of the reticular stroma of the tumor. The distribution of the bone lamellas did not follow the rules of statics.



Fig 8—Photomicrograph ($\times 80$) showing the distribution of newly formed trabeculae within the tumor. The old cortex is seen in the left upper corner. New trabeculae extend from the medullary surface of the cortex into the tumor tissue. Note the increase of reticular interstices of the tumor at the inner surface of the newly formed trabeculae. Tumor tissue entirely replaces the marrow.

Fig 9—Photomicrograph ($\times 200$) showing metaplastic new formation of non-lamellated bone trabeculae within the fibrous reticulum of the tumor. Van Gieson stain.

Hyperplasias of reticuloendothelial tissue, as well as primary tumors, are generally considered as bone-destroying lesions. Osteogenesis observed in such disorders has been thought to be a reaction to the osteogenic impulses occurring in the tumor or a sign of reparative tendencies on the part of the endosteum or the periosteum. To date, active bone formation as a specific property of these tumors has not been recorded because osteogenesis was mostly obscured by bone destruction due to the overwhelming destructive properties of these tumor cells. It will be shown later, however, that detailed roentgenologic and histologic investigations almost always reveal bone formation in reticuloendothelial tumors in addition to bone destruction if a sufficient amount of collagen or reticulum participates in the formation of the tumor. This is most frequent in late stages of the disease.

Cases like the present one are rare not only with regard to the excessive bone formation but also with regard to the nature of the underlying disease.

We are well aware of the fact that without autopsy no definite statement can be made concerning possible participation of internal organs in the disease process. The striking findings, however, necessitate the publication of this case, specifically because a review of references yielded no information concerning similar observations. Diffuse reticuloendothelial hyperplasias of internal organs and of the marrow as well as solitary tumors of the same origin, are well known. Disseminated nodular distribution of the foci of the disease has apparently not been recorded without coexisting lipoid granulomatosis.

The often cited case of Marckwald¹ in which multiple endothelial tumor of the bone marrow with bone formation was observed is not clearly understood. From the data and the illustrations given I was not convinced of the reticuloendothelial origin of the tumor. Chester² reported a case of lipoid granulomatosis with circumscribed sclerosis of several bones. Microscopic examination revealed that bone had been deposited in fibrous interstices of cellular hyperplasias. The deposits were present in abundance in places where the disease displayed signs of having healed spontaneously. Wassiljeff³ observed a case in which diffuse osteosclerosis of the skeleton and diffuse reticuloendothelial hyperplasia of the marrow were present. The latter case belongs to the group in which generalized osteosclerosis is associated with disorders of blood formation.

1 Marckwald, W. L. *Virchows Arch f path Anat* **141** 128, 1895

2 Chester, W. *Virchows Arch f path Anat* **279** 561, 1930

3 Wassiljeff, G., in Downey, H. *Handbook of Hematology*, New York, Paul B. Hoeber, Inc., 1938, vol. 4

SUMMARY

A case of a primary disseminated nodular reticuloendothelial tumor of the bone marrow with neoplastic growth of both the reticular and the endothelial components of this cellular system has been observed. Marked circumscribed bone formation was present in areas involved by the tumor. The newly formed bone tissue was mainly contributed by metaplasia of collagenous and reticular interstices of the tumor itself. Roentgen findings were suggestive of osteoblastic tumor metastases.

Multiple primary bone-forming reticuloendothelial tumor of the marrow is an extremely rare disease. I failed to discover any reference to a similar case in the literature at my disposal.

ALLOXANTIN

An Investigation of the Substance as Used in Experimental Production of Diabetes

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AND

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PHILADELPHIA

IN 1937 JACOBS¹ discovered that alloxan injected into normal fasting rabbits produced hypoglycemia and convulsions. To ascertain whether this action was due to alloxan itself or to one of its derivatives, he tested 12 other closely related substances, with negative results. Among the drugs was alloxantin. These negative observations were confirmed in dogs by Goldner and Gomori² in 1944. In the same year, however, Koref and associates³ reported that alloxantin injected intravenously into rabbits had an effect identical with that of alloxan. There was temporary hyperglycemia followed by marked hypoglycemia then permanent hyperglycemia. The histologic changes in the islets of Langerhans consisted of degeneration and necrosis with diminution in the number of beta cells. They further reported that, despite low blood sugar levels, none of their animals had hypoglycemic convulsions. If this were true, alloxantin might prove to be a good substitute for alloxan, because the latter, while its diabetogenic action is undisputed, has the disadvantages that in spite of close vigilance some of the animals succumb to hypoglycemic convulsions and, of those that survive many die within the next few days from hepatic and renal necrosis⁴. It thus seemed desirable to reinvestigate the diabetogenic action of alloxantin.

PROCEDURE

Thirty-five rabbits weighing between 2,000 and 3,020 Gm were given injections of alloxantin in the following manner. Six were given 1,000 mg each subcutaneously, 3 were given 800 mg each and 2 were given 1,000 mg each intraperitoneally, 6 received 100 mg, 15 received 150 mg and 3 received 200 mg per kilogram of body weight intravenously. A week later 3 of the rabbits given subcutaneous injections received intravenously 200 mg of alloxantin per kilogram

From the Clinical Laboratories, Jefferson Medical College Hospital

1 Jacobs, H R. *Proc Soc Exper Biol & Med* **37** 407, 1937

2 Goldner, M G, and Gomori, G. *Endocrinology* **35** 241, 1944

3 Koref, O, Vargos, L, Rodriguez, F H, and Telch, A. *Endocrinology* **35** 391, 1944

4 Herbut, P A, Watson, J S, and Perkins, E. *Hepatic and Renal Necrosis in Alloxan Diabetes in Rabbits*, *Arch Pathol* **41** 516, 1946

of body weight, and 3 of those given intravenous injections of 100 mg per kilogram of body weight and 5 of those that had received intravenously 150 mg per kilogram of body weight were each given a second injection of 150 mg of alloxantin per kilogram of body weight. The levels of blood sugar and blood nonprotein nitrogen were determined before the injection, and repeated determinations of blood sugar levels were made at approximately hourly intervals on many of the rabbits for the first twelve hours after the injection and on all the rabbits at irregular intervals during the two weeks of the experiment. Blood sugar, blood nonprotein nitrogen and blood cholesterol levels were determined on all the animals at the time of death. In the case of rabbits with permanently elevated blood sugar the urine was tested for sugar and acetone bodies. All surviving animals were killed in two weeks with a blow on the head. Immediately after death, tissues from the lungs, the liver, the kidneys, the adrenal glands and the pancreas were fixed in solution of formaldehyde U S P diluted 1:10 and in Helly's fluid,⁵ and sections of these were stained with hematoxylin and eosin. In addition, sections of the pancreas were stained by Bensley's method for alpha and beta granules.

Alloxantin is soluble in water or in saline solution in very small amounts. Heating a 1 per cent, 2.5 per cent or 5 per cent solution in water or in saline solution to 50 C did not increase the solubility appreciably. The relatively large crystals of alloxantin were therefore ground to a fine powder in a sterile mortar and the amount allotted to each rabbit was weighed separately and put into a sterile test tube. About 10 cc of sterile saline solution was added to each tube just before injection, and the consequent suspension was given relatively slowly. Since alloxantin precipitates out rapidly, it was necessary to turn the syringe continually while the drug was being introduced. In spite of this some of the powder precipitated out, and this was resuspended in 10 cc of saline solution and injected as before. To avoid blocking, we used a 16 gage needle for the subcutaneous and intraperitoneal injections and an 18 gage one for the intravenous injections.

RESULTS

Clinical Observations—Alloxantin injected subcutaneously had no clinical effect on the rabbits. If the drug was irritating, none of the animals cried or showed other signs of discomfort.

A few seconds after intraperitoneal injection of the drug the rabbits became stretched out, with their heads arched backward, and almost simultaneously underwent twitchings of the muscles of the back, the abdomen and the lower extremities. Two of these animals recovered, and 3 died in from five to fourteen hours after the injection.

Intravenous administration did not prove as irritating as anticipated, for only 2 of the rabbits cried, and that appeared to be more from fear than from pain. All animals thrashed about a little but not more than those which received alloxan. The veins that were used, however, became completely thrombosed a few seconds after the injection was completed. Of the 6 animals that received 100 mg per kilogram of body weight, 1 died during the injection and another three hours later. The rest showed no ill effects. Seven of the 15 rabbits that received 150 mg of alloxantin per kilogram of body weight died from a few minutes to nineteen hours after the injection. Most of those that survived the immediate effects but died

⁵ Helly's fluid is a modification of Zenker's solution in which, instead of 5 cc of glacial acetic acid, solution of formaldehyde U S P is used in the concentration of 5 per cent.

later merely became drowsy and listless and just before dying showed some jerky purposeless movements. One animal that died nine hours after the injection had severe pulmonary edema, with frothy pink fluid literally dripping from the nose and the mouth. In 2 rabbits typical hypoglycemic convulsions developed two hours and eighteen hours after the injection, which were readily controlled with subcutaneous injections of dextrose. Later permanent hyperglycemia developed in these animals. Two of the 3 rabbits that received an initial dose of 200 mg of alloxantin per kilogram of body weight died within five minutes after the injection, and 1 was found dead approximately four hours later.

The reactions to the second injections were similar to those following the first. One of the 8 rabbits that received 150 Gm per kilogram of body weight showed twitchings, crying and exuding of frothy fluid from the nose and the mouth five hours after the injection, and died shortly thereafter. The remaining 7 animals in this group showed no ill effects. One of the 3 rabbits that received a second injection of alloxantin in a dose equivalent to 200 mg per kilogram of body weight died at once, the others survived. Diabetes did not develop in any of the animals that received a second injection.

Chemical Observations—Following the initial injection there was transitory hyperglycemia followed by temporary hypoglycemia, then either transitory hyperglycemia with a return to normal in a few days or, in 2 rabbits, permanent hyperglycemia with excretion of sugar in the urine. The normal blood sugar levels varied from 70 mg to 102 mg, with an average of 91 mg, per hundred cubic centimeters. In forty minutes to five hours the levels rose to from 123 mg to 400 mg, with an average of 202 mg, per hundred cubic centimeters. The low blood sugar levels were reached in from five to fifteen hours and varied from 36 mg to 80 mg, with an average of 69 mg, per hundred cubic centimeters. The subsequent high levels were reached in from five to twenty hours and varied from 114 mg to 363 mg per hundred cubic centimeters. The blood sugar levels following second injections were not determined until twenty-four hours after the administration of alloxantin, at which time they ranged from 129 mg to 448 mg, with an average of 195 mg, per hundred cubic centimeters. After twenty-four hours the levels gradually declined until, within a week, they were all normal. The normal level of blood nonprotein nitrogen varied from 36 mg to 64 mg per hundred cubic centimeters. It remained unchanged in all but 2 rabbits. In one of these, which died twenty-four hours after the first injection, the level was 175 mg, and in the other, which died five hours after the second injection, it was 184 mg, per hundred cubic centimeters. The level of blood cholesterol was normal in all animals.

Gross Pathologic Observations—In 4 of the 6 rabbits that received alloxantin subcutaneously an abscess developed at the site of injection. The largest abscess measured 10 cm across, but the walls were collapsed, and there was only a little pus present, the overlying skin was a little stiff but showed no gangrene. One animal had a perinephric abscess on the right side, which was apparently directly continuous with the subcutaneous abscess. Except for slight accentuation of the lobular markings of the liver in 2 other rabbits, no other organic involvements were observed in this group.

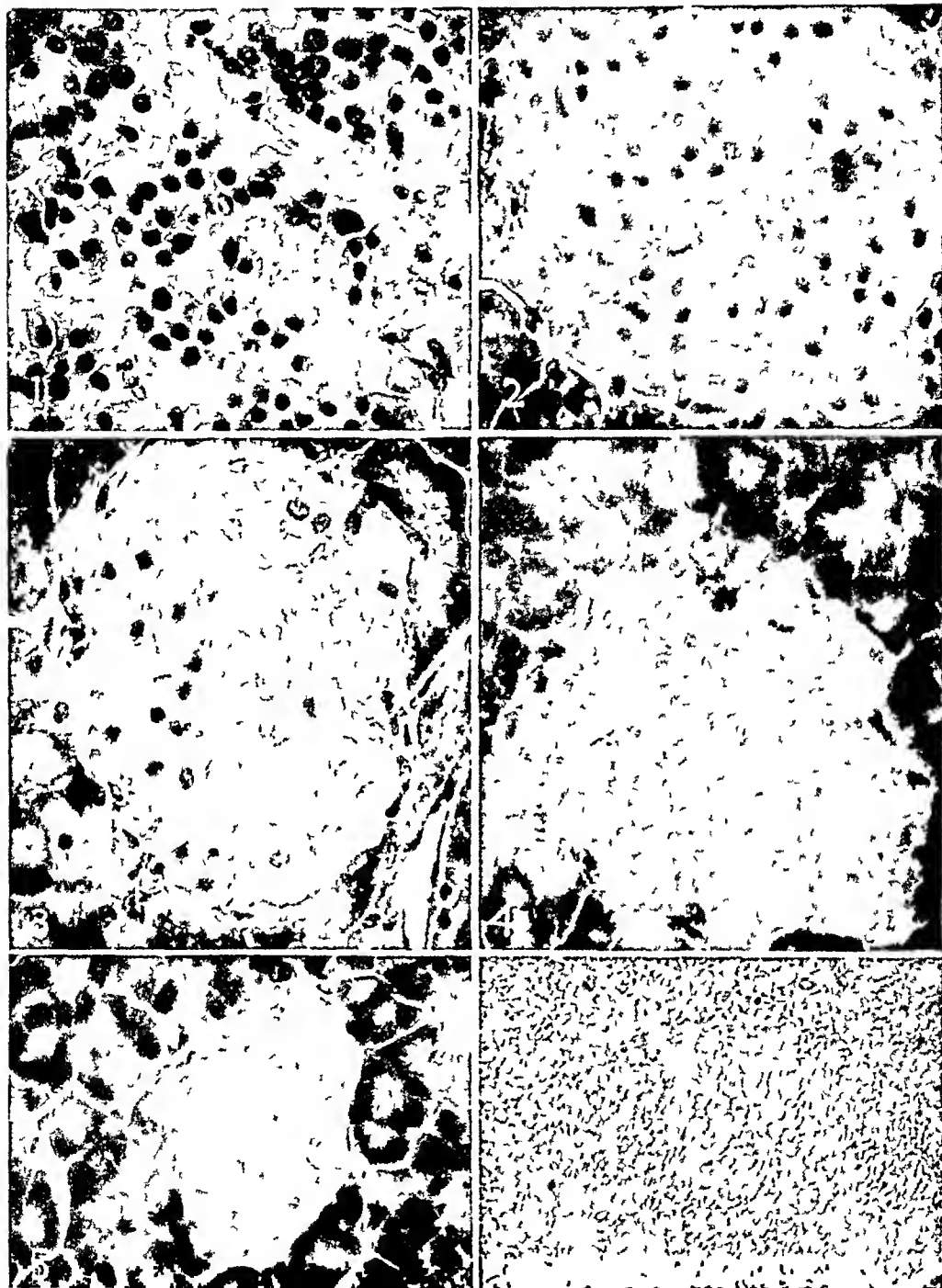
The 2 of the 5 rabbits receiving intraperitoneal injections that died within five hours disclosed general peritonitis. There was about 150 cc of fluid, together with dense but recent fibrinous adhesions of loops of the intestines. The rabbit that died at fourteen hours showed excess fluid but no adhesions. Of the 2 that survived and were killed in two weeks, one revealed fibrous adhesions between the liver and the right kidney, and the other was normal.

There were no gross pathologic changes in the 2 rabbits that died during the intravenous injection of the drug. In 10 animals that died within a few minutes to twenty-two hours after the intravenous injection there were severe pulmonary congestion, edema, hemorrhages, atelectasis and emphysema. As already mentioned, in 2 of the rabbits this was so severe that frothy pink fluid dripped freely from the nose and the mouth. In 3 animals that received 200 mg of alloxantin per kilogram of body weight the lungs actually appeared as if they were scorched. They were normal in 1 rabbit that died twenty-three hours after the injection and in all rabbits that were killed at the end of two weeks. The lobular markings of the liver were exaggerated in 3 animals that died seven, nine and twenty-three hours after the injection, but none showed hepatic necrosis. The liver was normal in all the other rabbits of this group. There were petechiae in the kidneys of 1 animal that died three hours after the injection and marked swelling and mottling of the cortex in another that died in twenty-two hours. The remaining organs showed no noteworthy changes.

Microscopic Observations—There were histologic changes in the islets of Langerhans in 11 of the 35 rabbits. Three of these were rabbits given intraperitoneal and 8 were rabbits given intravenous injections. The initial alterations were seen as early as forty minutes after the injection and consisted of pyknosis of a few cells with no visible change in the cytoplasm. The distribution of these cells varied. Sometimes they were scattered throughout the islets, at other times they appeared to involve only the central portion (figs 1 and 2). Within three hours after the injection some of the islets showed pyknosis of almost all the cells while others were less severely involved. By this time the cytoplasm was somewhat swollen and more dense. At fourteen hours the degenerating cells were definitely swollen and the cytoplasm granular, and by twenty-two hours the cells in the central portion showed severe degeneration progressing to complete necrosis. The nuclei were still pyknotic but were less heavily stained, or they disappeared entirely, and the cytoplasm was granular or vacuolated (fig 3). Often only a thin ring of cells filled with alpha granules was found at the periphery. In such islets beta granules were not apparent. They were, however, still present in those islets showing only degenerative changes. In one of the 2 rabbits in which diabetes developed and which were killed at the end of two weeks the islets were fewer, but in the other they were not. In each, however, they were reduced to about one quarter of the normal size, and the central portions of many of them were devoid of cells and filled with granular debris (fig 5). The cells at the periphery were normal and contained alpha granules. One mitotic figure was noted in a hypertrophied islet in one of these rabbits (fig 4). The same islet disclosed two tiny foci of detritus indicating that it had been previously damaged.

The lungs of the animals that died during the injection showed no changes except some atelectasis. All others that died within the first day showed moderate to severe congestion, edema, hemorrhages and varying degrees of atelectasis and emphysema. The vessels, however, disclosed no microscopic alterations. The lungs of an animal that died with pulmonary edema five hours after the second injection showed in addition foci of old pneumonia and thrombosis of the veins. The former consisted of plugs of fibrin surrounded by mononuclear cells. The septums were broad, and a few contained neutrophils and eosinophils. In these areas there were several medium-sized and small veins that were plugged with an admixture of fibrin and leukocytes. The arteries were not affected.

The livers of 5 rabbits disclosed in the central areas of the lobules varying degrees of degeneration advancing to complete necrosis. In one of these animals, dying at fourteen hours, the involvement was small, was located to one side of



(See legends on opposite page)

the central vein and consisted of simple degeneration progressing to necrosis of the liver cells with slight erythrocytic extravasation. In the remaining 4 animals, which died seven hours, nine hours, twenty-two hours and fourteen days after the injection, the lesion occupied as much as three fourths of the lobule and completely surrounded the central vein (fig 6). In some areas leukocytic extravasation was scanty, in others it was profuse. In all cases the hepatic cells immediately adjacent to the portal triads were intact. In the livers of the other 30 rabbits there were no significant alterations.

In only 1 animal were the kidneys abnormal. This rabbit received a single intravenous injection of alloxantin in a dose equivalent to 150 mg per kilogram of body weight. He died about twenty-two hours after the injection. Grossly, as already mentioned, the kidneys showed swelling and mottling of the cortices. Microscopically, this mottling was seen to consist of areas of severe degeneration and swelling of the renal tubules alternating with areas of dilatation of the lumens and sloughing of the tubular epithelium. These changes were practically confined to the proximal convoluted tubules. The lumens of the more distal portions of the nephrons contained granular and erythrocytic casts. All the glomeruli were swollen and contained focal areas of necrosis. The interstitial tissue disclosed edema and foci of detritus, nuclear fragments and leukocytes. The vessels were normal. The adrenal glands were normal.

COMMENT

Koref and associates³ reported that since alloxantin oxidizes readily it was kept in a vacuum until used. Perhaps this was due to their method of preparing the drug, for the commercially available alloxantin is supplied in an ordinary unsealed bottle with no precautions to prevent oxidation. Once the substance is in solution and exposed to air, oxidation as evidenced by a change to a pink or a red color occurs. Even with such exposure, however, the oxidation of alloxantin is not any more rapid than that of alloxan.

There is apparently no disagreement as to the irritating action of alloxantin. Although none of our rabbits that received subcutaneous or intravenous injections manifested any acute pain or violent general reaction, the aural vein used became thrombosed immediately on the completion of the injection. Intraperitoneally, too, the drug must have been irritating, for the animals stretched out to a maximum and underwent twitchings and even contractions of the muscles of the abdomen, back and the lower extremities. The 3 rabbits that died showed excess peritoneal fluid and 2 disclosed in addition extensive adhesive

EXPLANATION OF FIGURES

Fig 1—A portion of an islet of Langerhans with extreme pyknosis of most of the nuclei and condensation of the cytoplasm, in a section of pancreas from a rabbit that died four hours after intravenous injection of alloxantin. Hematoxylin and eosin, $\times 400$.

Fig 2—An islet showing extensive degeneration of all the central cells with beginning fading of the nuclei, in a section of pancreas from a rabbit that died twenty-three hours after intravenous injection of the drug. At the periphery of the islet there is an intact rim of alpha cells. Hematoxylin and eosin, $\times 400$.

Fig 3—An islet showing several cells with pyknotic nuclei and granular cytoplasm. In 2 of the cells the cytoplasm also shows hydropic degeneration. The rabbit died twenty-two hours after intravenous injection of the drug. Hematoxylin and eosin, $\times 400$.

Fig 4—A relatively hypertrophied islet wherein one cell is in mitosis. Note the two tiny foci of debris indicating the sites of previous necrosis. The rabbit had diabetes. Hematoxylin and eosin, $\times 400$.

Fig 5—A shrunken islet of a rabbit with diabetes. Note the intact peripheral cells and the large central area of detritus. Hematoxylin and eosin, $\times 400$.

Fig 6—Section of the liver of a rabbit that was killed two weeks after it had received an injection of alloxantin, the section shows complete necrosis of the central portion of a lobule. Hematoxylin and eosin, $\times 50$.

peritonitis At the end of two weeks fibrous peritoneal adhesions were present in 1 of 2 surviving rabbits A third indication of the irritating action of alloxantin, apparently on the capillaries, was the pulmonary edema that developed in all animals in which alloxantin was injected intravenously and which died early In 2 of these it was so severe that terminally frothy pink fluid dripped freely from the nose and the mouth

Repeated determination of the blood sugar levels of many animals revealed a curve identical with that obtained with alloxan except that the deviations from the normal were somewhat less pronounced There was temporary hyperglycemia followed by temporary hypoglycemia, then either permanent or transitory hyperglycemia Korei⁶ stated that in spite of low blood sugar levels none of their rabbits had convulsions Thus we could not corroborate for in our series there were only 2 rabbits in which diabetes developed, and each of these underwent convulsions

The histologic changes in the islets of Langerhans were similar to, but much less extensive than, those produced by alloxan Early there were pyknosis of the nuclei and condensation of the cytoplasm of a few or of many of the cells In the central portions of some of the islets the nuclei gradually disappeared and the cytoplasm became granular vacuolated and finally entirely desintegrated, leaving foci of complete necrosis Unlike what was noted with alloxan, however, there was always a peripheral rim of normal alpha cells remaining, and in none of the rabbits was there complete necrosis of all the cells In the animals in which permanent diabetes developed, the islets were reduced in size but not in number, and showed normal peripheral cells, some islets contained tiny central foci of detritus, which indicated areas of previous necrosis Hyalinization of islet cells was not seen, but one mitotic figure was encountered in a hypertrophied islet Since in this same islet there were two tiny foci of detritus, it seems fair to assume that the islet was the seat of necrosis and that its hypertrophy was due to active proliferation of the islet cells themselves The changes in alloxantin-treated animals might, therefore, be more comparable to those seen in human diabetes than are the lesions produced by alloxan, for in man necrosis of the islets of Langerhans has rarely been observed⁶, vacuolation of the islet cells has occasionally been noted, and the absence of any histologic changes is common⁷ Thus it may be that alloxantin is more akin to the diabetogenic factor in man than is alloxan

The side effects of alloxantin are somewhat different from those of alloxan Severe pulmonary congestion and edema appears to play

6 Duffy, E J Path & Bact 57 199, 1945

7 Warren, S The Pathology of Diabetes Mellitus, ed 2, Philadelphia, Lea & Febiger, 1938, p 31

an important role in alloxantin-treated animals and account for most of the early deaths. Some indication of more permanent damage of the lungs is evidenced by the finding in 1 animal of foci of organizing pneumonia and thrombosis of some of the veins. Alloxan, on the other hand, produced no pulmonary changes. The damage done to the liver by alloxantin was less frequent, less severe and of a different distribution as compared with that produced by alloxan. The latter caused necrosis, regeneration and fibrous tissue replacement of the peripheral portions of the lobules, whereas the former produced degeneration and necrosis of only the central portions. Only 1 of the animals treated with alloxantin disclosed damage of the kidneys, while alloxan produced extensive degeneration and necrosis of the tubular epithelium in many rabbits.

An evaluation of the two methods of producing experimental diabetes indicates that the use of alloxan is superior to that of alloxantin for the following reasons. Alloxan is easier to administer in that it is more readily soluble in water and isotonic solution of sodium chloride. It is less irritating, and its action is more dependable, that is, in adequate doses it will produce diabetes in 100 per cent of the animals, whereas alloxantin in nonlethal doses will produce diabetes in only a few. Undesirable side effects causing death in some of the animals occur with both and, while the sites of these actions vary with each substance, they are all equally fatal, leaving little to choose between the drugs from this standpoint.

SUMMARY

Alloxantin suspended in isotonic solution of sodium chloride was injected into rabbits subcutaneously, intraperitoneally and intravenously. Administered by the intravenous route it produced an effect similar to that of alloxan. There was temporary hyperglycemia followed by temporary hypoglycemia, then either transient hyperglycemia or, in a few of the animals, permanent hyperglycemia with excretion of sugar in the urine.

Histologic changes in the islets of Langerhans consisted of pyknosis of the nuclei, granular and hydropic degeneration of the cytoplasm and ultimately complete necrosis of the cells in the central areas. In the animals in which diabetes developed, the islets were decreased in size but not in number, and some showed remaining foci of detritus. Undesirable side effects consisted of thrombosis of the veins of the ears, severe pulmonary congestion and edema, and necrosis of the central portions of the hepatic lobules.

Although the diabetogenic action of alloxantin is unequivocal, it is not a good substitute for alloxan because it is difficult to administer, it is toxic, and nonlethal doses will produce diabetes in only a few animals.

REACTION OF RATS FOLLOWING INJECTION OF ANTI-RAT- HEART IMMUNE SERUM

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CHICAGO

THE role of immune and allergic reactions in the genesis of rheumatic fever has been studied by many investigators. In most of the experiments on animals a protein or a bacterial antigen has been injected that causes a variable circulating antibody response and also frequently an unknown degree of hypersensitivity of tissues. The experiments which will be presented were designed to produce an antigen-antibody reaction in which a specific antibody is supplied artificially in known amounts in order to minimize the number of experimental variables. Perhaps by this method lesions similar to those found in rheumatic fever can be produced.

Since rheumatic fever is a disease involving chiefly connective tissue, in the present investigation it was decided to prepare an antibody to rat connective tissue and then inject this into otherwise normal rats. Because there is considerable variation in the structure of connective tissue in various organs the hearts of rats were used as a source of connective tissue since it is the connective tissue of the heart that is significantly involved in rheumatic fever.

Longcope¹ inoculated animals (rabbits, dogs, cats and guinea pigs) intravenously, intraperitoneally or subcutaneously with egg white or horse serum or both and later gave intoxicating doses of the same material. He found swelling of the muscle fibers and focal infiltrations of lymphocytes in the hearts of the rabbits. The lungs of guinea pigs had small patches like bronchopneumonia with peribronchial infiltrations rich in eosinophil leukocytes. The livers of the rabbits and the cats had regions of necrosis, often periportal. There were also changes in the kidneys. Smadel,² Smadel and Farr³ and Swift and Smadel⁴ produced nephritis in rats by injections of antisera to rat kidneys. They noted changes in the hearts of some of their animals. They also injected rat heart antisera into rats as a

1 Longcope, W. T. J. Exper. Med. **18** 678, 1913, Arch. Int. Med. **15** 1079, 1915.

2 Smadel, J. E. (a) J. Exper. Med. **64** 921, 1936, (b) **65** 541, 1937.

3 Smadel, J. E., and Farr, L. E. J. Exper. Med. **65** 527, 1937.

4 Swift, H. F., and Smadel, J. E. J. Exper. Med. **65** 557, 1937.

control procedure but did not pay particular attention to the hearts of these animals because they were interested in renal changes. Rich and Gregory^{5a} injected horse serum intravenously into rabbits and found lesions resembling periarteritis nodosa. In some rabbits they found cardiac lesions of the specific rheumatic type with Aschoff bodies and valvular changes^{5b, c}. They pointed out that the tissue changes were the result of an anaphylactic type of hypersensitivity in contrast to the tuberculin type. As further evidence of the anaphylactic origin of the lesions of acute rheumatic fever, these observers^{5d} mentioned the basic similarity of the pneumonitis caused by sulfonamide hypersensitivity and that occurring in rheumatic fever.

MATERIAL AND METHODS

Preparation of Rat Heart Antiserum—The procedure was similar to that employed by Smadel² in the preparation of antikidney serum. With the rat under ether anesthesia, the heart was perfused with saline solution through the inferior vena cava. A perforation in an artery of the neck permitted the escape of blood and saline solution. In some instances the heart was removed while still beating and placed in saline solution until movement stopped. Each heart was then cut into small pieces, and these were washed by gentle pressure in repeated changes of saline solution in order to rid them of as much blood as possible. The small pieces were then ground with a mortar and pestle or with a Ten Brock grinder until the crushed tissues passed through a gauze filter several layers thick without leaving an appreciable residue. The ground cardiac tissue was then diluted to make a 10 per cent suspension and stored at 5 C until used.

Antiserum was prepared in rabbits by giving over a period of one month intravenous injections of a 10 per cent suspension of the antigen in amounts beginning with 1 cc and ending with 9 cc. Precipitin tests made according to the method recommended by Zinsser and Bayne-Jones⁶ revealed an antibody content that produced a precipitate with the rat heart antigen in dilutions of 1:4,000. Control precipitin tests in which the immune rabbit serum and normal rat serum were used indicated the presence of variable amounts of antibody to rat blood serum as well as that to rat heart tissue.

Immediate Effects of an Injection of the Antiserum in Normal Rats—Rat B9 (weight, 130 Gm) was given 2 cc of the antiserum, injected into the femoral vein. Respirations stopped in about thirty seconds, and the heart continued beating five minutes while artificial respiration was given. Three other rats, weighing between 100 and 250 Gm, were given 0.6, 0.5 and 0.3 cc intravenously. They died between five and thirty minutes after the injection. The ones that lived a

5 Rich, A. R., and Gregory, J. E. *Bull. Johns Hopkins Hosp.* (a) **72** 65, 1943, (b) **73** 239, 1943, (c) **75** 115, 1944, (d) **73** 465, 1943.

6 Zinsser, H., and Bayne-Jones, S. *A Textbook of Bacteriology*, New York, D. Appleton-Century Company, Inc., 1939, p. 932.

few minutes had labored and gasping respirations, and a clear serous fluid dripped from their nostrils—about 3 cc in 1 instance (These effects were not observed in control experiments in which normal rabbit serum was used)

Autopsy revealed in all rats conspicuous hyperemia, edema and hemorrhages of the lungs. A large amount of watery fluid was in the respiratory passages and there was an estimated 1 to 2 cc in the pleural spaces. Histologic examination of the lungs revealed hyperemia, edema and hemorrhages. The alveolar walls were greatly thickened by widely dilated capillaries. The erythrocytes in some of the small vessels were without distinct cell boundaries and formed dense hyaline masses like thrombi. In the air spaces were finely granular precipitates and strands of fibrin. Some were obscured by hemorrhages. Sections of liver, heart and kidneys revealed only slight hyperemia.

Effects of Repeated Injections of the Antiserum—Rats B10, B11 and B12 received repeated small injections of the antiserum intravenously, intramuscularly and intraperitoneally. Rat B10 received a total of 17 cc over seven days, B11 7.5 cc over fourteen days and B12 8 cc over twenty days. After each injection there were labored respirations, an outpouring of nasal secretion and symptoms interpreted as abdominal distress. The animals writhed as if in extreme discomfort and assumed unnatural positions. Turned on their backs, they made little effort to right themselves. The effects varied somewhat with the route of administration, the writhing being more prominent after intraperitoneal injection.

Histologic examination of the lungs revealed a combination of retrogressive changes and a proliferative reparative process. The lungs of rat B11 had marked thickening of the alveolar walls by fibrin-like material and connective tissue cells with large vesicular nuclei. Between the cells were delicate and coarse irregular strands of fibrin and hyaline material. These changes were seen also about many of the small blood vessels. Scattered throughout the lungs were variable numbers of phagocytes, some containing carbon pigment and polymorphonuclear leukocytes. There were hyaline changes of the walls of some of the smaller blood vessels. About some were eosinophil leukocytes. In addition there were variable edema and hyperemia, depending on the time since the last injection of antiserum.

Rats receiving intraperitoneal injections had a striking proliferative response of the peritoneum, which was revealed on the surfaces of the liver and the bowel. The thickened capsule of the liver was formed mainly by fibroblasts with large vesicular nuclei, with edematous, faintly fibrillar and granular interstitial tissue that stained poorly. There was a scanty infiltration of small round cells.

Rats B13, B14 and B15 received intracardiac injections of antiserum. In each case the injection was continued as the needle was withdrawn in order to let some of the antiserum escape into the pericardial space. Autopsy revealed proliferative changes of the epicardium and the pleura, essentially the same as those of the peritoneum of the rats described in the foregoing paragraph. There were also focal regions of necrosis of the cardiac tissue in the path of the injecting needle. Muscle fibers showed hyaline degeneration and fragmentation. Frequently portions of the cytoplasm were removed, so that only cell outlines remained. Hemorrhages, fibrin deposits and variable infiltrations of polymorphonuclear

leukocytes were also present. Control animals that were given injections of normal rabbit serum had only poorly defined regions of myocardial edema.

Except in animals receiving intracardiac injections, there were no consistent lesions of the heart that resembled Aschoff bodies.

COMMENT

The antisera obtained by using rat hearts as antigen when injected into rats intravenously in sufficient quantity (as little as 0.3 cc with some sera) produced fatal reversed anaphylactic shock regularly. This anti-rat-heart serum contained precipitins for rat blood serum elements, as demonstrated, and probably to other tissue not organ specific. Therefore, the effects following injection of the serum cannot be ascribed to antibody for a given tissue element, such as connective tissue, endothelial tissue, myocardial muscle tissue or plasma proteins, but must be considered the combined effects of antibodies to the various tissue elements present in cardiac tissue. Anaphylactic shock in rats has been produced only with some difficulty⁷ by the usual methods of sensitizing the animal with repeated inoculations of a protein and after a latent period giving a shock dose of the same protein. During this latent period circulating antibodies appear in the blood. When the shock inoculation is given, the antigen reacts with the antibodies, and anaphylactic shock results. In the experiments described here, however, the antibody was supplied artificially and reacted immediately with antigen which in this case is part of the normal tissues. The shock produced in either is similar. Both the Parkers^{7b} and Pratt^{7a} have described hyperemia, hemorrhages and edema of the lungs similar to the changes observed here. Congestion of the abdominal viscera, particularly of the intestine, also was noted.

Smadel² described a similar anaphylaxis-like reaction in rats with the administration of anti-rat-kidney serum. Histologic study of the tissue changes following these reactions was confined chiefly to the kidneys.

Since in most instances in the present study the antiserum was injected into the femoral vein, the first extensive capillary bed encountered was in the lungs. The antibody was probably "fixed" to the lung tissues and therefore was soon removed from the circulation. The resulting acute tissue reaction was analogous to the Arthus phenomenon with an antigen-antibody reaction causing extensive damage to the capillary walls, resulting in edema, hyperemia and hemorrhages. As suggested by Opie,⁸ should antigen and antibody meet within endothelial cells and form a precipitate within the cytoplasm,

7 (a) Pratt, H. N. *J. Immunol.* **29**: 301, 1935. (b) Parker, J. T., and Parker, F., Jr. *I. M. Research* **44**: 263, 1923-1924.

8 Opie, E. L. *I. Immunol.* **9**: 259, 1924.

the injurious reaction would be especially severe, and the result would be expressed in increased permeability of the blood vessels. The serums used in this experiment were antisera to normal rat tissue and therefore would produce an even greater reaction with tissue cells, such as endothelium, than would occur in the Arthus reaction produced in the usual way.

These experiments enabled a study of the effects of repeated antigen-antibody reactions involving given tissues. Usually anaphylactic shock can be produced only once in a given animal. Repeated reversed anaphylactic reactions were produced by injecting the anti-serum into rats B10, B11 and B12. The end result in the lungs histologically was a marked proliferative and reparative change with fibrosis and conspicuous thickening of the alveolar septums with some perivascular infiltration and hyaline changes.

By injecting antigen directly into various viscera and body cavities of sensitized animals other workers⁹ have produced essentially similar lesions.

There is a considerable similarity between the acute and the chronic pulmonary changes produced experimentally and the pulmonary changes seen in rheumatic fever. Epstein and Greenspan¹⁰ described in the early stages of rheumatic pneumonia congestion of the alveolar capillaries with considerable fluid passing into the alveolar septums and the interlobar spaces and with large mononuclear cells being desquamated into the alveoli. Later, as the changes progress, there are thickening and mononuclear cell infiltration of the interlobar septums, with resultant fibrosis and organization. Rich and Gregory¹¹ described similar pulmonary changes and pointed out the definite similarity between anaphylactic pneumonitis caused by drug or serum sensitivity and rheumatic pneumonitis. In the experiments described in this paper acute and chronic changes of the lungs of rats resembling the lesions described in human rheumatic pneumonitis were produced by reversed anaphylaxis.

Proliferative changes of serous cavities (peritoneal, pleural and pericardial) occurred in the cavities into which antisera were injected. This apparently was the result of a local antigen-antibody reaction and was analogous to the reparative proliferative lesions of the lungs occurring with the repeated reversed anaphylactic shock. This proliferative response to an antigen-antibody reaction is characteristic of the Arthus phenomenon⁹ and is further evidence of the potentials of immune reactions in the genesis of disease.

The focal lesions of the heart did not occur frequently enough to be ascribed to the effects of antisera.

9 Stenn, F. Arch Path **26** 244, 1938

10 Epstein, E. Z., and Greenspan, E. B. Arch Int Med **68** 1074, 1941

SUMMARY

Anti-rat-heart rabbit serum was prepared by injecting a suspension of ground rat heart into rabbits. This antiserum when injected intravenously in sufficient quantities into rats produced an anaphylaxis-like shock, including hyperemia, hemorrhages and edema of the lungs, and death. Repeated inoculation of rats with small quantities of the antiserum produced proliferative and reparative changes of the lungs similar to the lesions described as rheumatic pneumonitis. When the anti-rat-heart rabbit serum was injected into serous cavities of rats, it produced marked proliferative changes.

Case Reports

DUODENITIS WITH DIVERTICULUM AND ECTOPIC PANCREATIC TISSUE

BERNARD KALFAYAN, M D, BEIRUT, LEBANON

IN THE case to be presented, the condition of the patient was diagnosed clinically as a deficiency state resulting from pathologic changes in the stomach and the duodenum. In considering the nature of these changes, the presumptive diagnosis was peptic ulcer probably undergoing cancerous changes and gradually leading to pyloric stenosis. Roentgenologic studies failed to confirm this possibility. Autopsy revealed an extremely severe form of duodenitis, sharply limited to the distal two thirds of the duodenum, with duodenal diverticula and ectopic pancreatic tissue in the proximal third.

Inflammation occurring in an anomalous organ is not unusual, but the location, the extent and the nature of the lesions in this case were such that it was considered worth reporting.

REPORT OF A CASE

H. T., a married woman 33 years of age, was admitted, Oct. 13, 1943, to the Hospitals of the American University of Beirut. She complained of epigastric pain and intermittent vomiting, from which she had suffered for the past eight years, accompanied for the last two months by ascites and edema of the lower extremities. Her social and past medical histories were essentially irrelevant.

About eight years prior to admission she had an attack of severe diarrhea, with mucus, pus and a little blood in the stools accompanied with mild fever. The condition subsided in a month's time, but a few weeks later she began having attacks of mild epigastric pain and distention after meals, followed by nausea and relieved by vomiting. The epigastric distress began from fifteen to sixty minutes after meals, was never severe, had no relation to the type of food and was seldom relieved by alkalis. Occasionally she experienced hunger pains that were relieved by food. At the onset these attacks lasted several days, recurred once or twice a month and were worse during the colder seasons. However, she had no other complaints, and her general health remained fairly normal for five years.

Three years before admission she had daily spells of vomiting. The attack continued for a month then intermittently with progressive loss of weight. For the last three months she had amenorrhea and ascites with edema of the lower extremities. For the last two months she had a recurrence of diarrhea with pus and mucus and occasional tarry stools.

On admission the patient was afebrile but appeared seriously emaciated. In addition to the ascites and edema, she had a tender, reddish tongue. The heart sounds were weak, and the respirations were shallow but otherwise were normal. The white blood cell count was 7,000, with polymorphonuclears 86, lymphocytes 8 and large mononuclears 6 per cent. The red blood cell count was 4,220,000, the hemoglobin content, 10.9 Gm. per hundred cubic centimeters. There were no significant findings in routine and bacteriologic studies of the urine and the stool.

From the Department of Pathology, American University of Beirut

Two days after her admission a fever developed that lasted for a week, her temperature fluctuating between 38 and 39 C (100.4 and 102.2 F). With proper medical care the vomiting and the diarrhea stopped, yet she presented dysphagia, refusing everything by mouth, and, in spite of feeding by vein, she lost ground rapidly and died twenty days after admission.

Laboratory Data—Examination of the blood showed total plasma protein 25 mg per hundred cubic centimeters of serum (albumin 10, globulin 15) and urea nitrogen 27 mg, sodium 292 mg and chlorides 440 mg per hundred cubic centimeters of blood. The test for sugar tolerance revealed a gradual rise from a fasting level of 60 mg per hundred cubic centimeters of blood to 90 mg within two hours, at which time a trace of sugar appeared in the urine. The Wassermann, Kahn and agglutination tests of the blood gave negative results. Analysis of the gastric juice showed no free acidity even after injection of histamine phosphate, a test for occult blood was positive, a test for lactic acid was negative. The ascitic fluid (3.5 liters obtained by paracentesis) had the characteristics of a transudate, bacterial culture produced no growth.

Report of Roentgenologist—"The esophagus is free of defect. The stomach is moderately enlarged and contains a definite excess of mucus and secretion. Peristalsis and evacuation are sluggish. There is no demonstrable filling defect or niche suggestive of ulcer in the stomach. The duodenal cap is large and wide but shows no deformity or niche. The second portion of the duodenum is widened, and in both the second and the third portion there is stasis. The gastroduodenal curvature is of normal width. At six hours there is a slight gastric residue, but most of the opaque meal is seen in the lower small intestines and the cecum. At twenty-four hours the whole colon is outlined and shows no defect."

Autopsy—The body was preserved in the refrigerator and was examined fourteen and a half hours after death.

The stomach was slightly enlarged and contained excess mucus. The mucosa of its body was thickened and mamillated. The pyloric orifice appeared normal.

The suprapapillary portion of the duodenum was dilated (circumference 16 cm after formaldehyde fixation, its walls were thin, but the mucosal folds appeared normal. Three centimeters distal to the pylorus a patch of pancreatic tissue, 4.5 by 3 cm, was found incorporated in the anterior wall of the duodenum.

The head of the pancreas was firmly incorporated in the medial wall of the second portion of the duodenum. On cutting through it close to the papilla, a diverticulum was discovered, 8 mm in length.

On the same level with the duodenal papilla, a nodule, 1.0 by 0.5 cm, was felt in the antimesenteric side of the wall.

The infrapapillary portion of the duodenum down to the jejunal junction was converted into a rigid tube by cicatricial connective tissue and adhesions involving the neighboring organs. These changes increased in intensity as the duodeno-jejunal junction was approached. The mucosal folds were not seen. The lumen was gradually narrowed, with corresponding thickening of the walls, where the duodenum was crossed by the root of the mesentery, the wall measured 7 mm in thickness.

Eight centimeters distal to the level of the papilla, an indurated nodule, 1.5 cm long by 1.0 cm wide by 1.0 cm deep, was seen protruding out from the antimesenteric side of the wall. It contained pocketed pus.

Apart from the cecum, which showed congestion, the rest of the intestines appeared normal. The glands of the mesenteric root were moderately enlarged. The pancreas was atrophic and weighed 20 Gm only. The liver appeared fatty and contained in the left lobe a cavernous hemangioma 2.0 cm in diameter. The gall-

bladder was distended with half a liter of bile, its mucosa appeared normal. The biliary passages were patent. The adipose tissue was markedly depleted of its fat content. The blood was of a bright red color and was not clotted. There were no significant gross findings in the other organs.

Microscopic Examination—(a) Stomach. The mucosa was infiltrated with plasma cells, lymphocytes and large mononuclears. The deeper portions of some of the glands showed cystic dilatation. In the pyloric region however, the inflammatory changes were more pronounced, with partial destruction of glands and beginning fibrosis.

(b) Duodenum. The mucosa of the suprapapillary portion was infiltrated with round cells, but there was no destruction of epithelium.

The aberrant pancreatic tissue was of normal structure, though infiltrated with small round cells. It was separated from the mucosa by loose connective tissue containing a few of Brunner's glands, any trace of the muscle layer was lacking here. Externally it was covered by loose connective tissue containing small lymph glands and many dilated tortuous lymphatic vessels.

The diverticulum close to the papilla was made of mucosa and submucosa containing a few of Brunner's glands. There was a small islet of smooth muscle at its neck, otherwise no muscle layer could be seen. The lamina propria of this pouch was infiltrated with small round cells as was the rest of the mucosa of the suprapapillary portion. The pancreatic duct in this section was denuded of its epithelium.

The nodule at the level of the papilla was made up of densely packed small round cells and granulation tissue. It was covered by broken-down, degenerating muscularis mucosae—all that remained of the mucosa. The underlying muscle layer was partially eroded.

The infrapapillary portion of the duodenum showed extreme destruction of the mucosa. Epithelial structures were absent, and the wall was lined in different sections by thickened, cicatricial muscularis mucosae, by necrotic and granulating tissue or even by the fibrotic muscle layer of the duodenum. The submucosa and the serosa were extensively infiltrated with small round cells, with formation of lymph follicles. The muscle layer was infiltrated with these cells and showed marked destruction. The serosa was markedly edematous. All the layers showed cicatrizing granulation tissue.

The distal nodule was made up of a central focus of suppuration surrounded by inflammatory granulation tissue, which in turn was lined by hyalinizing cicatricial tissue. Few shreds of muscle were seen in its wall.

(c) Pancreas. This organ showed a mild chronic inflammation with cystic dilatation of some acini and patchy fibrosis.

(d) Other Organs. Small round cells were seen infiltrating the mucosa and the submucosa of the cecum. There was an old standing inflammation with fibrosis in the mesenteric lymph glands, although foci of polymorphonuclears were also seen. Multiple organizing thrombi were seen in the medium-sized veins of the lungs.

COMMENT

The two anomalies in this case were the diverticulum near the papilla and the ectopic pancreatic tissue.

Duodenal diverticula are not uncommon. Odgers,¹ quoting Grant and Linsmayer and giving the average of five other contributors, put the incidence in

1 Odgers, P. N. B. Brit J Surg 17: 592, 1930.

routine autopsies at 16, 33 and 25 per cent, respectively. Edwards² estimated the incidence as below 1 per cent. According to Illingworth,³ Odgers¹ and others, duodenal diverticula usually remain symptomless. Odgers¹ expressed the opinion that duodenal diverticulitis is rare because of the relative sterility of duodenal contents and the comparatively large size and the dependent position of the diverticular opening into the bowel.

The intimate relation of primary duodenal diverticula to the pancreas or to ectopic pancreatic tissue is well known. In this case, the presence of ectopic pancreatic tissue in the wall of the distended proximal third of the duodenum together with the absence of the muscle layer was indicative of the existence of a shallow diverticulum there, which had been flattened out as a result of the dilatation of this portion. Moreover, as duodenal diverticula are often multiple (Odgers), the finding of another diverticulum incorporated in pancreatic tissue is in support of this view.

Since the epithelial covering of these two anomalies was intact, there is no ground for supposing that infection started in either one of them. But the peculiarity of the case is not so much the origin of the infection as the extent and the location of it. The infection may have originated in the pancreas and spread to the duodenum by way of the pancreatic duct. The atrophy of the pancreas, the destruction of the epithelium of the pancreatic duct and the infrapapillary location of the severe inflammatory changes of the duodenum would all lend support to this view. Or the infection may have started as primary gastritis or duodenitis. According to Kellogg and Kellogg,⁴ Boyd⁵ and Judd and Nagel,⁶ chronic gastritis and chronic duodenitis are recognized clinical and pathologic entities, and frequently the two occur together. Whatever its origin, the infection once started probably became confined to one or more structural anomalies in the distal two thirds of the duodenum. The two nodules in the descending portion of the duodenum may well have been mucosal pouches with narrow outlets in which the infection either originated or became established, and from these pouches septic material was constantly washed out, causing severe and destructive inflammation of the surfaces with which it came in contact.

SUMMARY

After eight years of illness a 33 year old woman died with manifestations of nutritional deficiency. At autopsy the important lesions were confined to the distal two thirds of the duodenum and consisted of a granulating nodule at the level of the papilla and a suppurating nodule 8 cm distal to it, as well as extreme destruction of the entire mucosa with extensive chronic inflammation and fibrosis of the wall. There was a diverticulum in the immediate vicinity of the duodenal papilla, and there was a patch of aberrant pancreatic tissue in the wall of the proximal third of the duodenum, where the muscle layer was absent. There was simple chronic inflammation of the entire stomach and of the proximal third of the duodenum. The pancreas was markedly atrophic.

2 Edwards, H. C. *Lancet* **1** 169, 1934.

3 Illingworth, C. F. W. *Text-Book of Surgical Treatment*, Edinburgh, E. & S. Livingstone, 1943, pp. 478 and 479.

4 Kellogg, E. L., and Kellogg, W. A. *Am. J. Surg.* **21** 368, 1933.

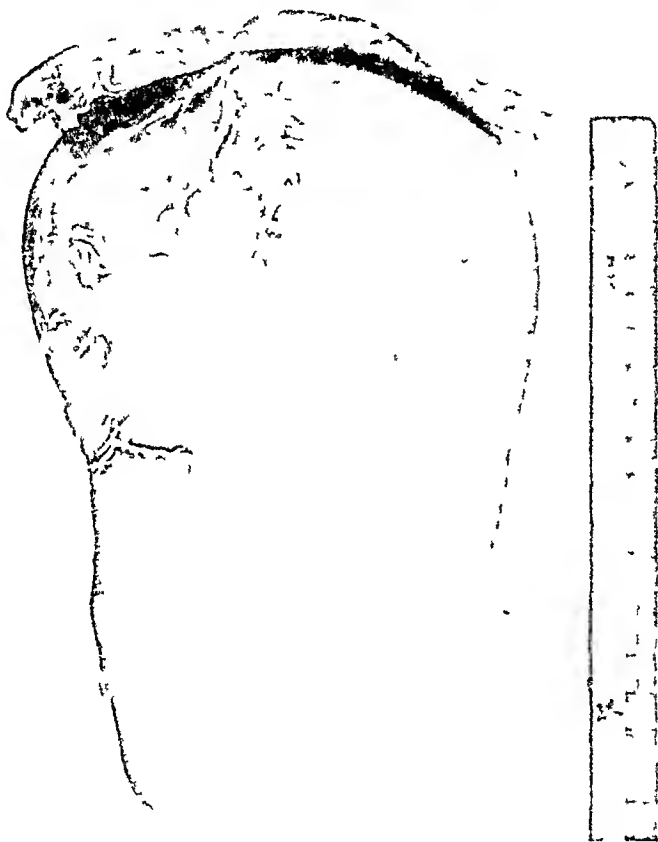
5 Boyd, W. *Text-Book of Pathology*, ed. 4, Philadelphia, Lea & Febiger, 1943, p. 566.

6 Judd, E. R., and Nagel, G. W. *Ann. Surg.* **85** 380, 1927.

COMPLETE ABSENCE OF THE LEFT LOBE OF THE LIVER

GEORGE G MERRILL, M D, BALTIMORE

COMPLETE absence of the left lobe of the liver is an extremely rare congenital anomaly. Only 1 case of it is described in the literature¹. The other reported cases of anomalies of the lobes of the liver were cases of anomalies of shape or of increased number of lobes, rather than of a decreased number. In the single previously reported



Anterior surface of the liver, showing complete absence of the left lobe

case of absence of a lobe there was a right lobe of normal size and shape, with normal bile ducts, normal blood vessels and normal microscopic appearance. There was no compensatory increase in the size of the lobe, in contrast to the case now described. The embryologic defect must occur at an extremely early stage of development, as

From the Johns Hopkins Hospital

1 Messing, A, and Montague, M F Anat Rec 53 169, 1932

the anlage of the right and that of the left lobe of the liver normally appear in the first few weeks of embryonic life, being seen in the 3 mm embryo

REPORT OF A CASE

The present anomaly is the only one of the kind seen in some 19,000 autopsies at the Johns Hopkins Hospital. It occurred in a 36 year old white woman who died of carcinoma of the right bronchus, which had metastasized to lymph nodes, the lungs, the ovaries, the right adrenal medulla and vertebrae. There were no metastases in the liver. Clinically the case was interesting because of the uncertainty regarding the primary origin of the tumor. The fact that the elongated right lobe of the liver extended downward so that its lower edge was palpable in the right lower quadrant of the abdomen led most examiners to regard this palpable abdominal mass as the primary tumor rather than as an unusually shaped normal liver.

The most striking features of the liver at autopsy were the absence of the left lobe and the elongation of the right lobe. There was no evidence of any left lobe ever having been present. The right lobe was greatly elongated, with its lower margin reaching down well into the right lower quadrant of the abdomen. It measured 22 cm in length, 13.5 cm in width and 7 cm in thickness in the upper third. The circumference at the point of maximal thickness in the upper third was 33.5 cm. The anterior surface was smooth. The superior surface and the ligaments were normal except for the absence of the left lobe. The caudate lobe was normal. The posterior surface of the liver had shallow impressions marking its relationships to the stomach, the duodenum, the colon and the right kidney. The gallbladder lay in a transverse position, with its fundus at the median border of the liver 10 cm above the lower margin of the right lobe. The neck of the gallbladder was 7 cm lateral to the fundus, and the cystic and common bile ducts extended laterally and slightly downward to reach the duodenum at about the same level as the lower margin of the fundus.

Histologically, the liver was entirely normal. There was no evidence of altered function of the organ either clinically or pathologically. No other congenital anomalies were found. No vascular abnormalities were apparent.

Obituaries

SIMON FLEXNER, M D

1863-1946

Simon Flexner remained for a time the last survivor of the group of physicians who acquired professional maturity during the early period of the Johns Hopkins Hospital when Welch, Osler, Halsted and Kelly collected about themselves younger men of conspicuous ability and transmitted to them their enthusiasm for investigation in medicine. The close association of Dr Flexner and Dr Welch is described in the biography of Welch by Flexner and his son.¹ They designate this early period "The Heroic Age of American Medicine." Dr Flexner's greatest contribution to American medical science was the organization and administration of the Rockefeller Institute for Medical Research, but the background of this accomplishment was his own development as pathologist and investigator.

Abraham Flexner, the distinguished brother of Simon, has told in his memoirs the history of their family. His father came to America from Germany in 1853 and his mother two years later. There were seven boys and two girls and their father was eager that his sons should be doctors, lawyers and scholars. He died when Simon was 19 years old, but his wish was amply fulfilled. Abraham Flexner has given a moving account of the steadfast courage with which his mother maintained her numerous family under adverse conditions.

Simon Flexner, who became Doctor of Science of Yale, Harvard and Princeton, Doctor of Laws of Hopkins and Cambridge and Fellow of Oxford and received honorary degrees from at least ten other universities, held no collegiate degree. He received, then 26 years old, the degree of Doctor of Medicine from the Louisville Medical School in 1889. This school had had as transient teachers, Daniel Drake, Samuel Gross, Austin Flint and Elisha Bartlett and Dr Flexner said in "A Half Century of American Medicine," in his day still bore the impress of these teachers.² Nevertheless, lectures followed one another in bewildering sequence during the long days of four winter months and were repeated precisely during a second year, at the end of which graduation with the degree of Doctor of Medicine was all but automatic. Of laboratory instruction there was none, and the dissecting room provided the one place where instruction might be regarded as practical.

1 Flexner, S, and Flexner, J T. William Henry Welch and the Heroic Age of American Medicine, New York, Viking Press, Inc, 1941.

2 Flexner, S. Science 8 505, 1937.

Chance, in accord with the usual meaning of the word, seems to have had an insignificant part in the development of Dr Flexner's career. Even before he left Louisville the urge to pursue a career in medical science was evident and he had published in local medical journals three papers in which he discussed the relation of some aspects of pathologic histology to clinical medicine. In one of these he describes Ehrlich's observations on the structure of the blood corpuscles, and in another he reviews several of Löffler's publications concerning the diphtheria bacillus and discusses its diagnostic significance.

"That fall" (1891), he says, "I came to Baltimore eager to work in Welch's laboratory. I had not attempted to practice, but had used Delafield's and Prudden's book,³ as well as other simple texts to teach myself a little pathology. My preparation was the most rudimentary". Dr Welch admitted the applicant to his course for graduates in medicine, though he did not seem particularly interested in the young man's desire to study pathology. Flexner spent all of his time in the pathologic laboratory and was able to complete a study which Dr Welch regarded as worthy of publication. Dr Welch's attitude toward him, he says, changed immediately, but this interest took a form that dismayed him. When he asked for permission to take Dr Welch's course in bacteriology, Dr Welch advised him not to take it. "There is no occasion to take the course. Study a problem." He followed this advice and abandoned his plan to return to Louisville and, as he says, eke out a living from pathology. Very soon (1892) he was appointed Fellow in Pathology and became, as he terms it, a sort of understudy to Councilman.

As Fellow in Pathology Dr Flexner, in association with Dr Welch, made a study of the histologic changes produced when animals were inoculated with the diphtheria bacillus and of the focal destruction of cells caused by soluble products of the micro-organism. When Councilman left the laboratory in 1892, Flexner was appointed as his successor. The industry with which Flexner developed his knowledge in the fields of pathology and bacteriology is well shown by the reports of the meetings of the Medical Society of the Johns Hopkins Hospital. Here doubtless the reputation of the younger members of the staff of the hospital was established. He presented many case reports of anatomic lesions, especially of those of tuberculosis and typhoid, bacteriologic observations (*Bacillus pyogenes filiformis*, nov. spec.), and notes on amebic abscess of the jaw, together with more elaborate studies in which the medical literature related to the subject was fully discussed. The routine bacteriologic examinations which accompanied

3 Delafield, F, and Prudden, T. M. A Handbook of Pathological Anatomy and Histology, New York, William Wood & Company, 1885.

autopsies were a significant factor in building up his comprehensive knowledge of the etiology and the pathology of disease. The detailed studies of terminal infections and of the etiologic factors of acute peritonitis which he published show with what meticulous care these bacteriologic observations were made.

A monograph on the pathologic changes caused by toxic protein substances ("The Pathology of Toxalbumin Intoxication") was published,⁴ in which emphasis was placed on the focal character of the lesions that were produced in parenchymatous organs especially the liver and lymphoid tissue, by the soluble toxin of the diphtheria bacillus and expressed the view that the lesions of human and of experimental diphtheria were identical. They were compared with the localized destruction of tissue produced by the poisons abrin and ricin, from the seeds of the castor oil plant. These substances, Ehrlich had shown were, like diphtheria toxin, capable of producing by immunization antitoxins that inhibited their injurious action. As an example of toxic proteins derived from the animal kingdom Flexner used the blood serum of one animal introduced into another of a different species. In addition to destruction of blood corpuscles and resulting hemoglobinuria he found localized death of cells in lymphoid tissues and in parenchymatous organs, followed in the latter by evidence of chronic change. It is noteworthy that, as a by-product of his study of toxalbumin intoxication, he described several experiments which forecast anaphylaxis nearly a decade before it was recognized by Richet. He said "Animals that had withstood one dose of dog serum would succumb to a second dose given after the lapse of some days or weeks, even when the dose was sublethal for a control animal." As Morgenroth has pointed out, Magendie, using egg white, made a similar observation.

During six years Dr. Flexner lived in the Johns Hopkins Hospital as one of the resident staff and, like his associates of the period, including Dr. Osler, remained in residence after he had become one of the senior members of the medical faculty. The accompanying portrait of him is reproduced from a photograph made at that time. In 1893 he was abroad and studied pathology in Prague and in Strassbourg, for a time as a student of von Recklinghausen. During the summer months several years later, working in the laboratory of Jacques Loeb in the Marine Biological Laboratory at Woods Hole, he studied the regeneration of the nervous system of planarians and the structure of anomalous forms produced experimentally.

Dr. Welch had a keen interest in pathologic anatomy and a wide knowledge of the subject, but he was primarily concerned with the etiology and the pathogenesis of disease. In the universities of the

4 Flexner, S. Bull. Johns Hopkins Hosp. 6:259, 1897.



SIMON FLEXNER, M D
1863-1946

United States general pathology and experimental pathology have not been separated as organized departments from pathologic anatomy, though this procedure has been widely followed in Continental Europe. Pathology has included the functional as well as the anatomic aspects of the subject. When Dr. Flexner was promoted to a full professorship before he left the Johns Hopkins University he received what may have seemed to him the paradoxical title of "professor of pathological anatomy."

Two events significant in reference to Dr. Flexner's subsequent career occurred before he left Baltimore. He was designated by the State Board of Health of Maryland to investigate an epidemic of cerebrospinal meningitis that occurred in mining towns in the mountainous district of Western Maryland. Information about 120 cases was collected, and two autopsies were performed. It is noteworthy that "*Micrococcus lanceolatus*," already found in association with lobar pneumonia, was at this time regarded as the probable cause of cerebrospinal meningitis, and in exudate obtained from the meninges Flexner found diplococci within and about phagocytic cells.

In the period immediately following the war with Spain, at a time when the United States Army was engaged in suppressing the Filipino revolt, a commission consisting of Dr. Flexner and Dr. Lewellys Barker was appointed under the auspices of the Johns Hopkins University to make a study of the diseases of the Philippine Islands. J. M. Flint, T. P. Gay and John W. Garrett accompanied the commission as voluntary workers. A report on the diseases prevailing in the neighborhood of Manila was made, but the most important result of the undertaking concerned the cause of bacillary dysentery. Strains of the bacillus of dysentery brought back from the Philippine Islands were the means by which, as the result of investigation by many observers, it became evident that the disease was caused by two forms of the micro-organism since known as the "Shiga" and "Flexner" types. Later, at the Rockefeller Institute with Sweet he showed that the soluble toxin obtained from the Shiga type of the dysentery bacillus when introduced into the circulating blood of rabbits reproduced the characteristic intestinal lesions of human dysentery.

The almost immediate influence of Welch's laboratory on the development of pathology in this country is shown by the appointment of Dr. Councilman to the professorship of pathology at Harvard University and a little later by that of Dr. Flexner to the professorship at the University of Pennsylvania. Flexner assembled in Philadelphia a group of able young assistants, including Pearce, Bunting, Yates and Noguchi. The latter came unexpectedly as the result of a casual meeting in Japan preceding Flexner's visit to the Philippines. The activity of this laboratory during three years is indeed astonishing.

Flexner continued his studies of toxalbumins and the reactions of the immunized animal to them. He directed Noguchi to a highly successful study of the nature and the mode of action of snake venoms. He made experimental studies of pancreatic disease, begun in Baltimore and later continued in New York. He demonstrated a fat-splitting enzyme in foci of fat necrosis. He undertook experiments to define the conditions under which hemorrhagic necrosis of the pancreas is produced, and he showed later that the necrosis which develops when bile is introduced into the pancreatic ducts is referable to the bile salts. He described hitherto unrecognized thrombi produced by agglutination of red blood corpuscles. He planned to write a textbook of pathology and had begun the preparation of the illustrations for it. Had he remained in Philadelphia, he would doubtless have fulfilled this purpose.

Dr. Flexner supervised the organization of the Ayer Clinical Laboratory of the Pennsylvania Hospital and was its first director. Longcope as resident pathologist was the first of a succession of able pathologists who while associated with this laboratory made significant contributions to pathology and clinical medicine.

Simon Flexner, in 1903, married Helen Whitall Thomas, a member of a family of Baltimore intimately associated through her father and her sister with the development of Johns Hopkins University and Bryn Mawr College. As an author she has upheld the scholarly tradition of her family. Their children are William Welch Flexner, professor of mathematics, and James Carey Thomas Flexner, an author who collaborated with his father in writing the biography of Dr. Welch.

Mr. John D. Rockefeller, in 1901, gave a fund to promote research in medicine, to be spent within a period of ten years. The Rockefeller Institute for Medical Research was established under the guidance of an advisory board, which ultimately became the board of scientific directors. Of this board Dr. Flexner became a member. During two years the fund was expended in small grants in aid of medical research. In the second year Mr. John D. Rockefeller Jr. informed the board that his father had assigned to the institute a much larger sum, again to be spent within a subsequent period of ten years. Part of this fund was designated for the purchase of land and the erection of a laboratory building in New York. Dr. Flexner accepted the directorship of the institute. He studied physiologic chemistry for a time in the laboratories of Emil Fischer and of Salkowski in Berlin and then, pending completion of the new laboratory, began work with a small staff in two houses temporarily converted into laboratories. Significant events in the development of the Rockefeller Institute for Medical Research were the opening of the original laboratory building in 1906, the establishment of a hospital for clinical investigation, the inaugu-

ration of a laboratory of animal pathology at Princeton and the establishment of a department of plant pathology at the same place. The institute in New York increased greatly in size and scope.

The dominant purpose of Dr. Flexner's later career was the success of the institute as a center of fundamental and applied research, broadly included in the domain of the medical sciences. He personally sought out and pursued fruitful problems for investigation and by his own example pointed the way to much of the later work of the institute in pathology and microbiology.

The prompt and widespread recognition which the Rockefeller Institute for Medical Research received was in no inconsiderable part the result of Dr. Flexner's studies concerning the control of epidemic cerebrospinal meningitis. His attention had been directed to this subject while he was still in Baltimore, but epidemics of the disease that occurred in New York in the winter of 1904 and the spring of 1905 gave the problem immediate urgency. Approximately 3 of 4 of those who were attacked died. He was soon able to confirm the observation that the disease could be reproduced in monkeys by bringing the meningococci into contact with the meninges. The changes produced resembled those that occurred in man though the quantity of meningococci necessary to cause death was considerable and there was, he believed, scant multiplication. The experimental disease of the monkey afforded favorable opportunity to determine the value of serum obtained by immunization of the horse. Monkeys were protected when the serum was directly introduced into the subdural space by spinal puncture. Although this procedure had been employed in the treatment of the disease, its value was uncertain. With Jobling, Dr. Flexner published the results of intraspinal injections of the serum in 47 patients with meningitis, of whom only 12 died. On the basis of these observations they advised a wider trial of the antiserum. Later they found convincing evidence of its value and recorded the results of the treatment of 393 patients, of whom three fourths recovered. If treatment was begun early in the disease, the results were more favorable, of those who received subdural injections from the first to the third day of its course, only 16.5 per cent died. An analysis of the data concerning 1,300 patients who were carefully observed during treatment was published five years later, and the results were essentially the same as those previously recorded.

Studies of the transplantation of tumors were published by Flexner, Jobling and Menten in the first of a series of monographs issued by the Rockefeller Institute. The current studies of the period were in great part concerned with the conditions under which tumors of mice could be transmitted from one animal to another. The tumor of the rat studied by Flexner and Jobling was regarded by them

as an embryoma which at first had a simple carcinomatous structure but which with repeated transplantation assumed the characteristics of adenocarcinoma. Known as the Flexner-Jobling tumor, it has been perpetuated in laboratories in many parts of the world and has been the object of much experimentation.

Dr Flexner published in 1910 the first of a series of studies of poliomyelitis, and these were the earliest of many important studies of virus diseases made at the Rockefeller Institute. They began like the studies of epidemic meningitis with the transmission of the disease to monkeys. Landsteiner and his associates had shown that poliomyelitis could be transmitted to these animals by intraperitoneal and intracerebral inoculation and that the agent was conveyed by material that had passed through a Berkefeld filter. Flexner and Lewis, a few months later, confirmed these observations. Dr Flexner's interest in poliomyelitis centered about its pathogenesis, with especial reference to the mode of the body's elimination of the infectious agent, the manner in which the agent made its entrance into the normal host and the route by which it reached the meninges and the central nervous system. The virus was demonstrable only by the inoculation of monkeys with the production of the disease, and answers to any questions concerning it were dependent on this laborious procedure. He and his co-workers, Clark and Amoss, demonstrated that the disease of monkeys was almost identical with that of human beings. Gastrointestinal administration of infected material was seldom followed by the disease, but animals were readily infected if the same material was applied to the mucosa of the nose. Flexner and Amoss described experiments which indicated that the virus passed along the olfactory nerve to the olfactory bulb and thence to other parts of the central nervous system.

Poliomyelitis was produced when infected material was inoculated into any part of the nervous system, but it seldom occurred when infected material was introduced into the circulating blood. The meninges and the choroid plexus formed a barrier between blood and cerebral substance, but this barrier could be overcome with mildly irritant aseptic fluids, such as normal monkey or horse serum or isotonic solution of sodium chloride, which when brought into contact with the meninges by intraspinal injection inflamed or even slightly altered the integrity of the meninges and the choroid plexus.

Dr Flexner seems to have assumed that the conditions that had favored his own research would promote that of others. He has outlined his conception of the administration of research. "This meant in practice choosing promising young men, affording them suitable opportunities for work under little or no direction and retaining over a term of years those who showed the greatest aptitudes in finding

themselves and dealing successfully with the problems on which they were engaged" "Only an exceptional person, of course, is gifted with the power to extend knowledge, but a much larger number of persons can, under direction, add to the sum total, both kinds of ability were utilized by the growing institution. The power of the more gifted was enlarged through the use of the less, and the incidental training secured by the latter became a valuable asset in the educational expansion of the country."

Dr Flexner's wide knowledge of medical science with especial reference to public health and his demonstrated ability to solve problems requiring investigation established confidence in the soundness of his judgment. His advice was often sought by educational institutions that were contemplating changes in their organization. He was an earnest advocate of the "full time" plan of clinical teaching. Medical schools asked for his aid when they were attempting to find the best available persons to fill vacant professorships. When an epidemic of plague threatened the Pacific Coast, he was appointed by the national government a member of a commission to find means for its control. He was a member of the State Council of Health of New York, and for many years its chairman.

He served during a period of years as a member of the board of trustees of the Rockefeller Foundation and also of that of the Carnegie Institute of Washington. Shortly after his retirement from the directorship of the Rockefeller Institute of Medical Research he was appointed Fellow of Balliol College of Oxford and Eastman visiting professor. In his later years he renewed his association with the Johns Hopkins University as a member of its board of trustees.

Dr Flexner's career includes a period which begins with the return of medicine of the United States to the current of European science and ends with a time in which research has become a dominant feature of the medicine of this country. He has had an outstanding part in this development.

EUGENE L. OPIE

Notes and News

Appointments, Etc—Fritz Levy, formerly pathologist to the Davis Memorial Hospital, Elkins, W Va, is now pathologist to the Veterans Administration Facility, Huntington, W Va

R A Moore, professor of pathology, Washington University School of Medicine, St Louis, has been appointed acting dean

Kenneth Goodner, of the International Health Division of the Rockefeller Foundation, has been appointed professor of bacteriology and immunology at Jefferson Medical College, Philadelphia

At the Medical College of Virginia, Richmond, George Z Williams has been promoted to professor of pathology, in the department of bacteriology and parasitology R L Thompson has been appointed associate professor and William A Summers assistant professor

Commander H M Zimmerman (MC), associate professor of pathology at Yale University and recently executive officer of the United States Naval Medical Reserve in the Pacific area, has been appointed chief of the laboratory division of Montefiore Hospital, New York

Death—Ernst Freund, biochemist, founder of the first hospital clinical laboratory in Austria, originator of the Freund-Kaminer test for cancer, died in London June 2, 1946, at the age of 82

Award—The Alvarenga Prize has been awarded to William H Feldman, of the Mayo Foundation, in recognition of his studies on the chemotherapy of tuberculosis

Society News—The 1947 meeting of the Society of American Bacteriologists will be held in Philadelphia during the week beginning May 11, under the presidency of James Craigie, of the University of Toronto The secretary-treasurer is L W Parr, George Washington University, Washington, D C

Institute of Pathology—The Rhode Island Hospital, Providence, has established an institute for clinicopathologic service to other hospitals in the state

Needs of War Devastated Libraries—Medical and scientific books of the last decade and periodicals are greatly needed Shipments should be sent prepaid to American Book Center, care of the Library of Congress, Washington 25, D C The center depends on gifts from individual donors and institutions

The National Institute of Health—Applications for grants-in-aid from investigators in the field of pathology will be considered Applications should be addressed to the Chief, Research Grants-in-Aid Office, National Institute of Health, Bethesda 14, Md

Since the announcement, June 3, 1946, of the publication of a journal to be called *Research in Medical Science*, unforeseen circumstances have interrupted the program While publication has had to be abandoned for the present, it is hoped that the National Institute of Health will be able to carry out the original plan at a later date

A pathologic study section has been formed under the research grants-in-aid program of the National Institute of Health The basic purpose of this section is to foster research in general pathology The section consists of Paul R Cannon, as chairman, W A DeMonbreun, W D Forbus, Harry Goldblatt, J S McCartney, A R Moritz, Arnold Rich, J F Rinehart and H P Smith as members and R D Lillie, National Institute of Health, as secretary The section met Aug 16, 1946 in Bethesda

Books Received

DISEASES OF THE RETINA By Herman Elwyn, M D, senior assistant surgeon, New York Eye and Ear Infirmary Pp 583, with 170 illustrations, 19 in color Price \$10 Philadelphia and Toronto The Blakiston Company, 1946

The retinal diseases are considered under eight headings diseases from disturbances of circulation, diseases from vascular malformations, hereditary degenerations, inflammatory diseases, tumors, retinal detachment, developmental anomalies, radiation injuries The descriptions and discussions are marked by careful competence The writing is clear and concise There are many short, well chosen bibliographies The illustrations, selected from many sources, are uniformly excellent It is an attractive book that meets well the present special and general need of a complete and systematic presentation of the diseases of the retina

HUMAN TORULOSIS A CLINICAL, PATHOLOGICAL AND MICROBIOLOGICAL STUDY WITH A REPORT OF THIRTEEN CASES By Leonard B Cox, M D (Melb), M R C P (Edin), F R A C P, and Jean C Tolhurst, M Sc (Melb) Pp 149, with 67 illustrations Price, 25 shillings Melbourne Melbourne University Press, 1946

Thirteen new Australian cases of torulosis of the nervous system are reported in detail with illustrations All phases of torulosis, spontaneous as well as experimental, are considered on the basis of the cases studied and of a thorough review of the literature in question There is a comprehensive bibliography The monograph is a valuable contribution to the study of torulosis

THE JOHN AND MARY MARKIL FOUNDATION Annual Report, 1945 Pp 135 New York (14 Wall Street), 1946

MEDICAL JURISPRUDENCE AND TOXICOLOGY By John Glaister, J P, D Sc, M D, F R S (Edin), of the Inner Temple, barrister-at-law, etc, Regius professor of forensic medicine, University of Glasgow, formerly professor of forensic medicine, University of Egypt, Cairo, and medicolegal consultant to the Egyptian Government Eighth edition Pp 691, with 222 illustrations, 89 in color Price \$8 Baltimore Williams & Wilkins Company, 1945

The impression left with the reader of this eighth edition of a well known text is that contrary to a statement in the "Preface," it has not been "fully revised" Through the years the definition and clarity of the photographs have progressively deteriorated in the several editions to a point where the captions are now sometimes essential for the identification of the object portrayed

Much remains to be desired concerning the presentation of the pathology of trauma The illustrations are of scant assistance to the descriptions of gross anatomic findings, and histopathology is generally neglected Physiologic mechanisms are either ignored or inadequately elucidated It is questionable whether "cardiac failure due to inhibition induced by swimming while the progress of digestion was in active operation" can be a satisfactory certification of the cause of death

No information is included concerning the methods of identifying metallic and powder residues in and near wounds in the investigation of deaths caused by gunfire No mention is made of the effect of hydrogen ion concentration on the preservation of spermatozoa in cases of rape The chemistry of the blood of the right and that of the left side of the heart is not discussed in relation to the diagnosis of death by drowning

The section on toxicology, nearly a third of the text, is a compendium of brief clinical summaries and descriptions of chemical tests for various toxic agents, without discussion of pathologic or biochemical mechanisms Acetylsalicylic acid poisoning, a primary respiratory alkalosis, most certainly will not be cured by the administration of alkali

The illustrative cases of crimes make entertaining reading, but it is doubtful whether the intricacies of British and Scottish law are of value to American readers

ARCHIVES OF PATHOLOGY

VOLUME 42

SEPTEMBER 1946

NUMBER 3

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EPIDEMIC POLIOMYELITIS

Some Pathologic Observations on Human Material

JOSEPH A. LUHAN, M.D., Ph.D.

CHICAGO

THE BASIS of this study was a series of 13 fatal cases of poliomyelitis coming to necropsy at the Cook County Hospital during the epidemic of poliomyelitis that occurred in Chicago and Cook County in 1943. During this period permission to make a necropsy was obtained in 15 cases diagnosed as cases of poliomyelitis, but the first of these proved to be one of toxic encephalopathy associated with pneumonia, and another, meningococcic septicemia with very early or minimal cerebral leptomeningitis.

During 1943 there were 1,262 cases of poliomyelitis reported in Cook County, with 109 deaths, the case fatality rate being almost 8.6 per cent¹.

CLINICAL FEATURES OF THE DISEASE (SEE TABLE 1)

The patients in this series ranged in age from 4 to 34 years, with a median age of 8 years.

The duration of the illness varied from three to twenty-one days, with a median duration of six days. However, death occurred from two hours to twelve days (median, nineteen hours) after admission to the hospital.

The neurologic examinations of most of these desperately ill patients were superficial, so that it was not possible to correlate carefully the topography of the histologic lesions with the localization of the paralysis. Clinically, the patients in this series all showed "bulbar" involvement, but this was recorded as severe on admission in only 7 cases. Seven patients were observed to have no obvious paralysis of the extremities on admission to the hospital. Respiratory failure predominantly of the spinal type with intercostal paralysis was observed in 3 subjects. Four patients were treated in a Drinker respirator. Three showed terminal hyperthermia. Cerebrospinal fluid obtained by spinal puncture in 6 cases revealed cell contents ranging from 100 to 480 cells per cubic millimeter.

This study was aided by a grant from the National Foundation for Infantile Paralysis.

From the Department of Neurology and Psychiatry, Loyola University School of Medicine, and the Neuropathological Laboratory of the Cook County Psychopathic Hospital.

¹ Vital Statistics Bulletin, Yearly Report, Illinois Department of Public Health, 1943.

TABLE 1—Summary of the Clinical Features in This Series of Cases of Poliomyelitis

| Case and Patient's Initials | Age (Yr.), Sex and Color | Sequential Mode of Onset | Total Duration of Illness | Duration After Admission to Hospital | Duration of Bulbar Symptoms | Clinical Evidence of Spinal Paralysis on Admission | Comment on Clinical Course |
|-----------------------------|--------------------------|---|---------------------------|--------------------------------------|-----------------------------|---|---|
| 1 W K | 10 M White | Fever, listlessness, abdominal pain, then bulbar symptoms | 7½ days | 10 hours | 3+ | Arflexia, patient able to move extremities | Terminal hyperpyrexia (107.1 F, rectal) |
| 2 F D | 10 M White | Sore throat, fever, then difficulty in swallowing | 4 days | 25 hours | 3+ | Questionable left-sided foot drop, patient moved all extremities, deep reflexes 1+ | Patient became stuporous and had nystagmus, terminal hyperpyrexia (109.6 F, rectal) |
| 3 T C | 20 M White | Sore throat, vomiting, headache, bulbar symptoms 12 hours before admission | 5 days | 5½ hours | 2+ | No obvious paralysis, both knee jerks and right ankle jerk absent | |
| 4 T R | 6 M White | Vomiting, diarrhea, fever, remission 3 days then difficulty in swallowing and fever (dromedary type) | 6½ days | 6½ hours | 4+ | No apparent paralysis, deep reflexes all present | Patient stuporous, with body tremors, pupils dilated, involuntary movements of eyeballs |
| 5 F D | 18 F White | Headache, stiff neck and vomiting, next day severe vertigo | 6½ days | 4½ days | 3+ | None, deep reflexes present, abdominal reflexes absent | Interoctal paralysis and weakness of right arm developed later; patient became irrational, shortly before death temperature was 107 F (rectal), pulse rate 155, respirations 40 |
| 6 B K | 8 F White | Difficulty in swallowing, temperature of 106 F (oral), restlessness | 7 days | 6 hours | 3+ | Slight weakness of lower extremities | Nystagmus, circulatory collapse developed with marked cyanosis, terminal temperature, 105 F (rectal) |
| 7 H W | 7 M White | Fatigue, restlessness, nasal speech, next day stiff neck, dysphagia | 6 days | 10 hours | 0+ | No apparent weakness of extremities | Patient in Drinker respirator for 7 hours before death |
| 8 I I | 6 M White | Headache and repeated vomiting for 3 days; abdominal pain a few hours before admission | 7 days | 2 hours | 1+ | No gross paralysis, in extremities, deep reflexes (except biceps jerks) and abdominal reflexes absent | Temperature 103.8 F (rectal), respirations 2, pulse rate 100 on admission; patient had marked difficulty in breathing |
| 9 W T | 5½ M White | Listlessness, next day sore throat and fever, remission for a few days, then stiff neck, fever, weakness of arms (dromedary type) | 9 days | 13 hours | 1+ | Marked weakness of both upper extremities although deep reflexes were still present | On second hospital day marked bulbar symptoms developed, and patient was placed in Drinker respirator but failed rapidly |
| 10 R M | 4 M White | Headache, fever and vomiting next day difficulty in swallowing | 11 days | 23 hours | 3+ | No paralysis all deep reflexes brisk, as were abdominal reflexes | Shortly before death rectal temperature 104 F, respirations 40, pulse rate 110 |
| 11 T P | 23 M White | No history of mode of onset available | 21 days | 12 days | 1+ | Slight generalized weakness of extremities, marked interoctal paralysis | Patient very dyspneic on admission and placed immediately in respirator; at one time was able to remain outside the respirator for 27 minutes |
| 12 R D | 9 F White | Fever (102 F), headache three days later vomiting and dyspnea | 6 days | 2 days | 2+ | Interoctal paralysis, no note of paralysis of limbs made | Patient, an achondroplastic dwarf, placed in respirator a few hours after admission |
| 13 I W | 34 F White | "Intestinal influenza," abdominal pain diarrhea for two days remission, on 7th day dyspnea, dysphagia, coma | 7 days | 4 hours | 2+ | Deep reflexes "diminished to absent" but patient was in deep coma | Patient died after 9 hours in deep coma had had a convulsive seizure 2 hours after admission |

GROSS PATHOLOGIC CHANGES

The extraneural pathologic changes included well marked swelling congestion or hyperplasia of lymph nodes of the intestine and the mesentery in 6 patients. Twelve patients showed varying degrees of pulmonary congestion and edema, but only 1 had frank pneumonia (bronchopneumonia of the lower lobe of the right lung). One patient presented verrucous mitral endocarditis, without any embolic features or evidence of chronic congestive decompensation.

The brain appeared edematous to a slight or a moderate degree in 12 cases. Hyperemia of the cord and the brain stem was regularly encountered, but in only 1 case (7) were petechial hemorrhages seen within the neuraxis, and these were encountered in the thoracic part of the spinal cord.

HISTOLOGIC CONSIDERATIONS AND OBSERVATIONS

The main histologic features of human poliomyelitis are well known, but within recent years the work of a number of investigators, particularly that of Bodian and Howe,² has shown that the histologic distribution of the lesions may yield valuable clues concerning the mode of entry of the virus and its subsequent propagation and dissemination within the central nervous system. Examination of the present neurologic material was therefore undertaken with topographic relationships as one objective. Also, it seemed advisable to add a record of certain peculiarities of localization and nuances of histologic reaction seen in this sampling of the 1943 Chicago epidemic to the ample literature for whatever it might contribute to identification of the virus on anatomicopathologic grounds.

In the present series of cases, blocks from representative cortical areas were studied: the anterior prefrontal convexity, the basilar region of the frontal cortex immediately adjacent to the olfactory tract, premotor areas (Brodman 6), the motor cortex, particularly the area gigantopyramidalis (FAY of von Economo and Koskinas) near the vertex or in the paracentral lobule, the post-central cortex, the superior and inferior parietal lobules, the occipital cortex, including Brodmann's area 17, the hippocampal gyrus and region of the cornu ammonis, the gyri bordering on the sylvian fissure, and the island of Reil. These were merely isolated blocks, and so admittedly a good deal of the cortex remained unstudied, moreover, in the first 3 cases, one block was taken from the motor area of one side only (in addition to other cortical blocks) and the remainder of the brain discarded before this study was contemplated. Cresyl violet or toluidine blue, hematoxylin and eosin, and Weil stains were routinely used on sections cut from material embedded in paraffin and pyroxlin (nitrocellulose) (soluble cotton [20 to 25 per cent water]).

There were only 5 cases (3, 4, 5, 8 and 10) in which fairly complete olfactory bulbs and tracts were received for examination. Partial serial sections of these bulbs and tracts showed normal appearances. In all of these cases the medulla oblongata was severely involved.

In 12 of the 13 cases the gigantocellular motor area was involved by some degree of interstitial cellular infiltration with or without unmistakable perivascular cuffing. In the remaining case (3) only one small paraffin-embedded block was available from the left motor area. This appeared normal except for a few subcortical perivenous accumulations of mononuclears and scavenger cells.

² Howe H. A. and Bodian, D. Neural Mechanism in Poliomyelitis, New York Commonwealth Fund 1942.



FIGURES 1 TO 3
(See legends on opposite page)

It is possible that if more blocks through the motor area of this brain had been studied, typical cortical infiltrative changes might have been found

In severity these cortical infiltrates were rated as minimal in 5 cases, mild in 4, and prominent and unmistakable in the remaining 3 (figs 1 and 2). I should venture to guess that if a quick survey of the sections of the motor area were made as in routine histologic study one could easily miss the insignificant and scattered involvement of the motor area in about half of the cases. In 3 instances of minimal involvement only serial sectioning of the block revealed the changes, although in most cases the first section of the gigantocellular area encountered disclosed the inflammatory reaction. The disappearance of interstitial infiltration as soon as an adjacent cytoarchitectural area was reached was striking (fig 3).

Interstitial cortical infiltrations were found only in the gigantocellular area except in 1 case (9), and that presented the most pronounced involvement of the motor area in the whole series. The site of these other foci of infiltration of cortical tissue was in the hippocampal region, where they occurred in the form of occasional tiny cellular foci and borderline perivascular infiltrations in the upper cortical layers, underlying slight mononuclear infiltration of the leptomeninges, not far removed from moderately severe poliomyelitic involvement of the substantia nigra at this level.

The interstitial infiltrates in the motor cortex were often associated with mild mononuclear infiltration of the regional leptomeninges and small subcortical perivascular mononuclear and scavenger cell infiltrations. However, the leptomeningeal reaction was not limited to the motor area, it was seen particularly well developed in 1 case (4) in which mild discontinuous mononuclear infiltrations were found in the hyperemic and edematous leptomeninges over the frontal, temporal and parietal cortex.

Slight perivascular infiltration was noted in other cortical regions in addition to the motor area in 3 cases, in 2 instances the hippocampal region was affected, and in a third, the premotor cortex.

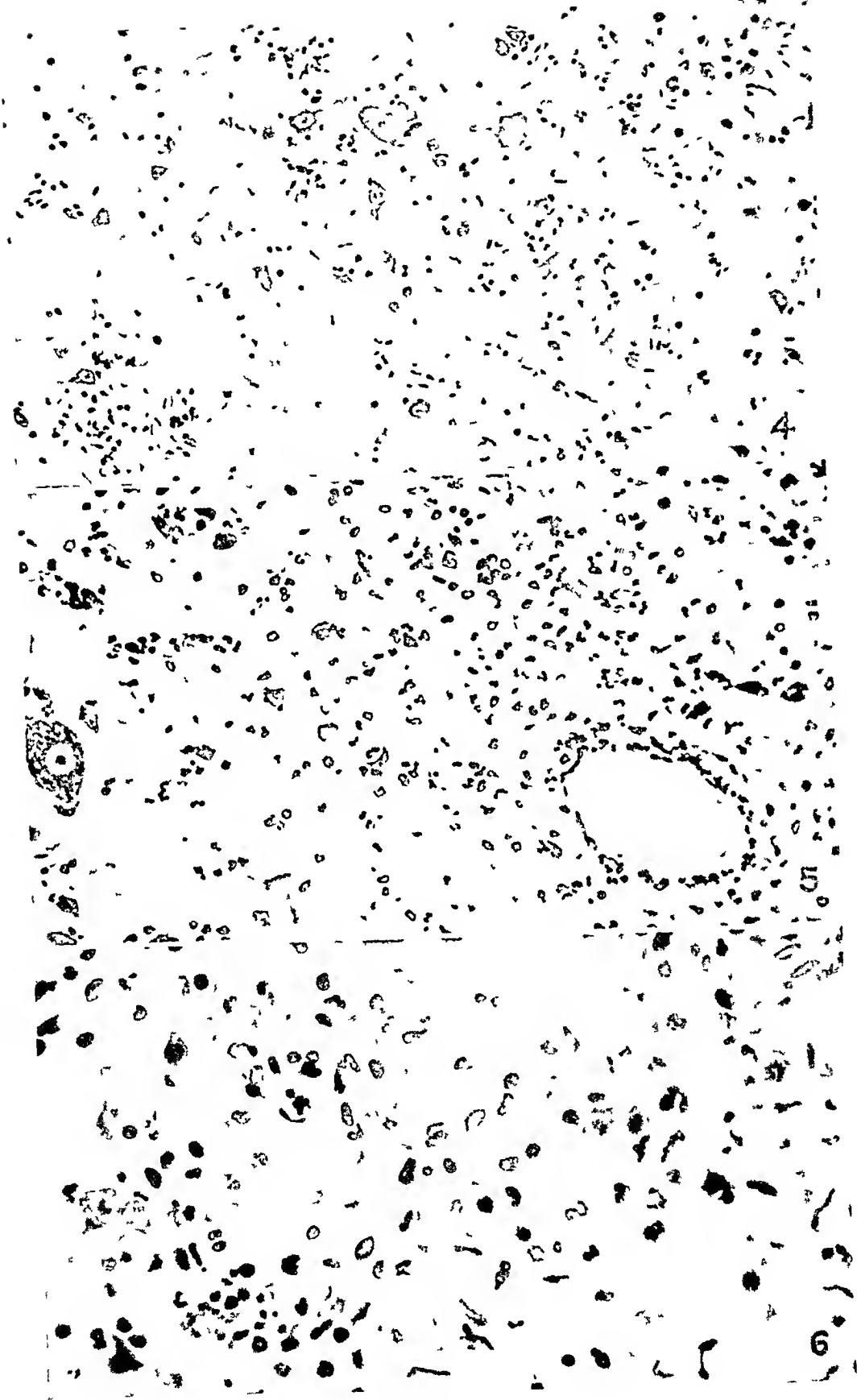
The intraparenchymal cellular infiltrates in the motor cortex were often predominantly microglial in nature (fig 4) except in a few cases where polymorphonuclear cells morphologically typical of leukocytes were conspicuous, presumably indicative of an early process (fig 5). In other instances a mixed polymorphonuclear and rod cell infiltrate was found (fig 6). The infiltrations were both focal and diffuse and were situated for the most part within the third to the fifth cortical layers. Occasional neuronophagic pictures were encountered, proportional to the severity of the interstitial infiltrate. These sometimes involved Betz cells (fig 7) but more often implicated small ganglion cells within the motor area. Sometimes the ganglion cells showed morphologic abnormalities without microgliosis or cellular infiltration of their immediate neighborhood (fig 8).

EXPLANATION OF FIGURES 1 TO 3

Fig 1 (case 1)—A typical minimal intraparenchymal infiltrate in the motor area of the cerebral cortex. Cresyl violet, $\times 150$.

Fig 2 (case 9)—Marked poliomyelitic involvement of the right motor area. Cresyl violet, $\times 120$.

Fig 3 (case 9)—Photomicrograph showing the poliomyelitic inflammation sharply limited to the motor area (*M*). The adjacent posterior central gyrus (*PoC*) is uninvolved. Cresyl violet, $\times 14$.



FIGURES 4 TO 6

(See legends on opposite page)

Most estimates of the comparative severity of inflammatory reactions within different regions of the neuraxis are relatively crude unless the tremendous task of counting cells and abnormal formations in serial section is undertaken as a basis for comparisons. One should distinguish in poliomyelitis between (1) perivascular infiltrations, (2) intraparenchymal cellular infiltrations and (3) histologic appearances of varying degrees of damage or destruction of ganglion cells.

Even when one disregards the relative degree and importance of these different components of inflammatory reaction and considers the severity of the reaction as a whole, one is still merely attempting to assay the intensity of pathologic change per area or volume of nervous tissue—which neither expresses the total volume-density of the inflammatory process nor assesses the biologic value of the part affected.

A quantitative appraisal of the total number of ganglion cells wiped out in the inflammatory process would probably disclose that the average cellular destruction for this present series among the given areas was highest in the cervical part of the spinal cord.

Considering the severity of all the parenchymatous manifestations as a whole (perivascular infiltration, infiltration of tissue and destruction of ganglion cells), one finds that the medulla oblongata was more severely involved than the cervical part of the spinal cord in 3 cases, was about as severely involved as that part of the cord in 6 instances and was less heavily implicated than the cervical part of the cord in 4 cases. Perivascular infiltration was usually more pronounced in the brain stem, particularly in the region underlying the lower part of the floor of the fourth ventricle, than in the cord. The severity of the parenchymatous process on the average diminished in a rostral direction from the medulla or the cervical part of the cord in most cases. The cord (especially the cervical part) and the brain stem, including the red nucleus and the substantia nigra, were more or less severely involved in most cases, the subthalamus and the hypothalamus were moderately implicated, the thalamus and the lenticular nuclei were relatively slightly involved (mainly by sparse perivascular infiltrations), and the cortex was usually unaffected except for slight discontinuous and selective implication of the motor area. The posterior part of the hypothalamus, as a rule, was more densely involved than the anterior part. Parenchymatous (in contrast to leptomeningeal) inflammation of the cerebellum was conspicuously lacking except within the roof nuclei and the dentate nuclei. The dentate nuclei, studied in 12 cases, showed interstitial lesions and destruction of ganglion cells in 11 cases and only perivascular infiltration in 1.

A crude sampling of the comparative total inflammatory intensity per area was attempted in the following manner. The usual 0 to 4 plus estimates of

EXPLANATION OF FIGURES 4 TO 6

Fig 4 (case 1)—High power view of a diffuse cellular infiltration, chiefly microglial, in the fifth layer of the motor cortex. Cresyl violet, $\times 180$.

Fig 5 (case 12)—Numerous polymorphonuclear leukocytes apparently streaming into the nervous tissue (motor cortex) from the small vessel. Cresyl violet, $\times 290$.

Fig 6 (case 9)—High power view of an infiltrate in the fifth layer of the motor cortex of the left hemisphere. Note the admixture of polymorphonuclear leukocytic and microglial (rod cell and polyblastic) forms in the infiltrate and the swelling of capillary endothelium. Cresyl violet, $\times 535$.

the severity of the visible pathologic alterations were made (figs 9 and 10) for a number of sections at various levels through a given region ascribing the same weight to perivascular infiltration and infiltration of tissue as to cellular destruction, and these averaged for all the brain and cord specimens for each area. Since these were biased because of varying discontinuity of the disease process,

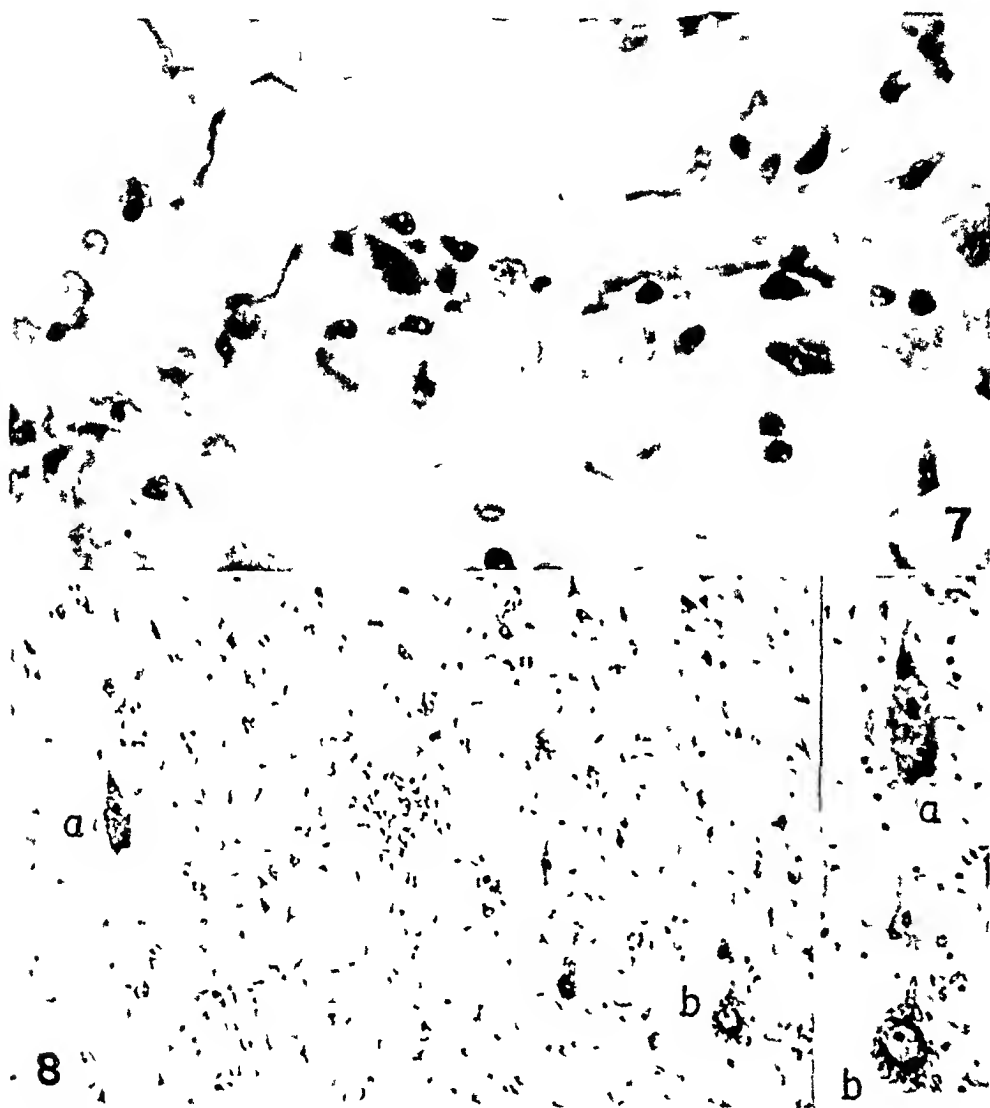


Fig 7 (case 2)—Microglial proliferation and coffin formation neuronophagia of a Betz cell in the motor cortex. Cresyl violet, $\times 600$

Fig 8 (case 12)—Focus of microglia as well as mammal diffuse cellular infiltration in the fifth layer of the motor cortex, the Betz cell marked *a* is severely damaged, in contrast with the normal-appearing cell *b* in the same field. Cresyl violet, $\times 125$ and $\times 225$

a further adjustment was made for severity per total area (thus only a fraction of the total area of the motor cortex showed, say, 1 or 2 plus change), and the final numerical average estimates (from 0 to 4 plus) were multiplied by 10

This gave the values for certain areas selected for comparison shown in table 2. Such numerical estimates connote much greater accuracy of observation than actually was possible. If visible neuronophagia and liquefaction necrosis had been selected as the criterion of severity, the cervical part of the cord would have a much higher rating than the rest of the neuraxis, but since it is probable that ganglion cells in the vicinity of a cellular exudate may be functionally damaged without showing gross staining abnormalities, the total density of cellular accumulation was used instead as the determining factor in the estimate. Where there is smoke, there is fire.

As previously mentioned, the densest perivascular infiltrations within the neuraxis in poliomyelitis tend to occur in the floor of the fourth ventricle (fig 11). In the midbrain the red nuclei and the substantia nigra are usually the most severely involved (fig 12). In the pons the locus ceruleus and the tegmental reticular formation generally are the most hard hit (fig 13). Sometimes considerable involvement of one or both facial nuclei occurs, with neuronophagia (fig 14). However, the basis pontis is almost unaffected in most instances, a circumstance difficult to explain if the virus travels along pyramidal pathways, unless the cells of the nuclei pontis are peculiarly immune to the virus. In the medulla the nuclei underlying the floor of the fourth ventricle, irrespective of

TABLE 2—*Comparative Intensity of the Total Visible Pathologic Alterations in Certain Regions*

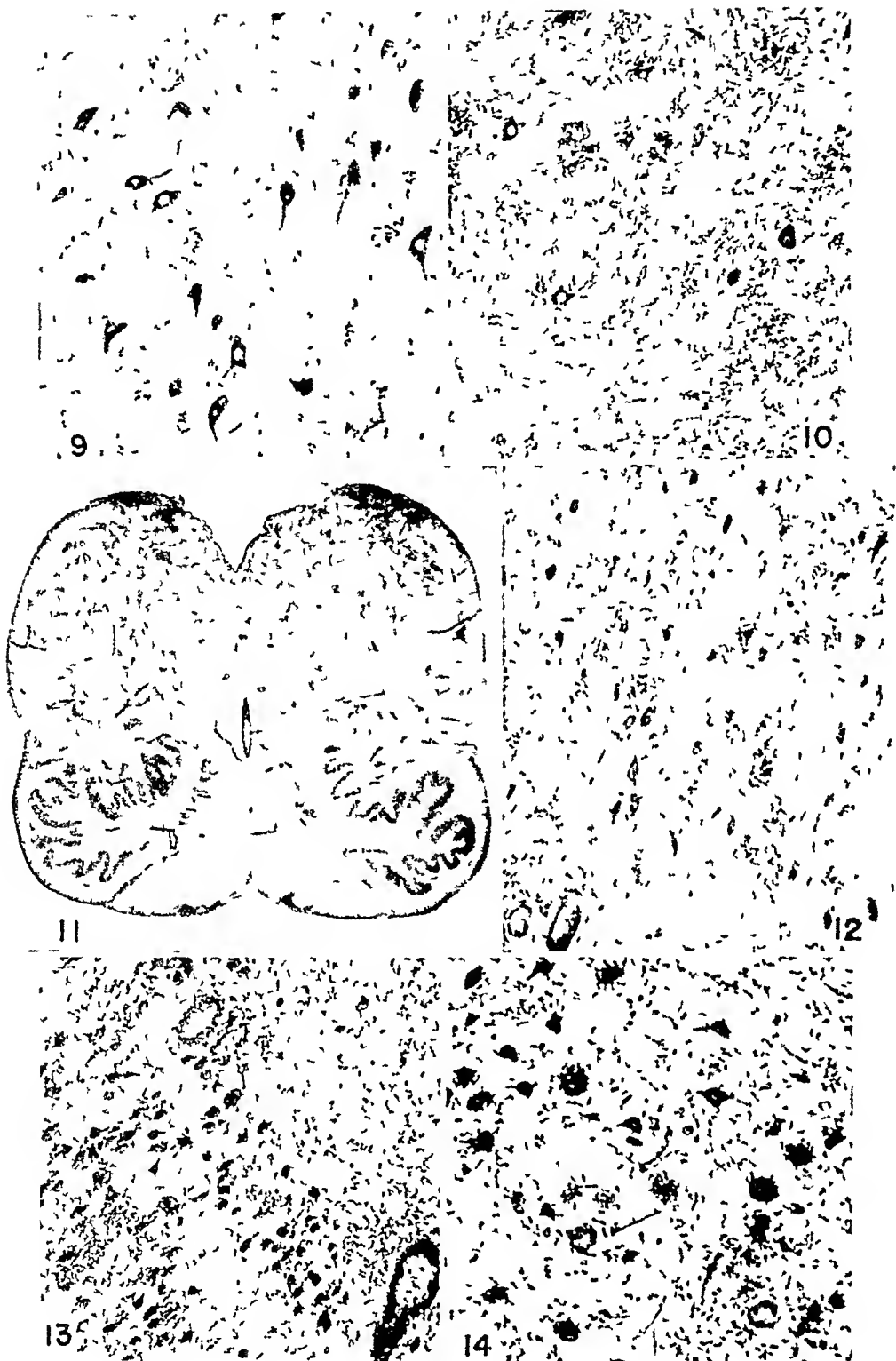
| | | | |
|-----------------------------|----|---------------------------|----|
| 1 Cervical part of the cord | 28 | 8 Sacral part of the cord | 12 |
| 2 Medulla oblongata | 26 | 9 Subthalamus | 10 |
| 3 Pontile tegmentum | 22 | 10 Hypothalamus | 10 |
| 4 Thoracic part of the cord | 18 | 11 Dentate nucleus | 7 |
| 5 Red nucleus | 18 | 12 Motor area | 3 |
| 6 Lumbar part of the cord | 18 | 13 Thalamus | 3 |
| 7 Substantia nigra | 17 | 14 Lenticular nucleus | 2 |

their function as motor or sensory, are prominently affected, but the reticular formation is the most uniformly involved, usually by diffuse cellular infiltration. The nuclei ambiguæ are more or less implicated in every case, although the actual cellular destruction is only moderate.

An interesting observation is the tendency of the interstitial infiltrate to assume compact, nodular formations in the dentate nucleus (fig 15). When the inferior olivary nucleus presents any significant intraparenchymal cellular infiltration (which is rarely encountered, in contradistinction to perivascular cuffing in its vicinity), the infiltrate is similarly nodular. These nodular formations in the olives were particularly noticed by Marinesco and co-workers³ in their study of the 1929 Rumanian epidemic. Occasionally a conspicuous nodular focus may be encountered in the nuclei pontis, a region singularly free from inflammatory foci. Thus, when certain cellular groups giving rise to the cerebellar peduncles are involved, the physical aggregation of the intraparenchymal infiltration tends to assume a distinctive form.

Certain vagaries of the disease process may be noted within the spinal cord. In 1 case (2), in the white matter of the thoracic and upper lumbar segments of the cord there were scattered minute foci of perivascular necrosis and micro-

³ Marinesco, G., Manicatide, M., and State-Draganesco. *Ann Inst Pasteur* 43: 223, 1929.



FIGURES 9 TO 14

(See legends on opposite page)

glial proliferation, a form of leukoencephalitis (fig 16) In another case (1) there was more posterior than anterior poliomyelitis in the lumbar segments of the cord, and at the first sacral level posterior poliomyelitis was conspicuous in the absence of involvement of the anterior horns (figs 17 and 18) Occasionally the inflammatory process was so severe, particularly in the upper cervical region of the cord, that small foci of rarefaction of the ground substance within the gray matter were produced, and pigmented cells were seen (cases 1 and 5, fig 19) However, apart from such tiny foci no demyelination was encountered

In 5 cases (2, 5, 9, 10, 13) of 9 in which the cord material was sufficiently complete at all levels to permit comparison, the newest process was found in the lumbar, sacral or lumbosacral segments of the cord An inflammation was interpreted as being fairly recent when generous numbers of polymorphonuclear leukocytes were seen in the perivascular and interstitial exudates and participating in neuronophagia This view is based on experimental studies⁴ In another case (3) there was progressive decrease in the severity of the inflammatory reaction from the cervical portion of the cord down, with the most recent lesions in the thoracic as opposed to the cervical part of the cord, and no lesions were found below the tenth thoracic segment Therefore, if the hypothesis of spread via axonal channels is adopted, there was some evidence for an infection descending within the cord in 6 of 9 cases

A general perusal of the evidence available from the topographic severity of the histologic lesions and from the clinical features leads me to classify the poliomyelitis in 3 cases (9, 11 and 12) as a primary spinal type with subsequent ascent of the inflammatory process, that in 9 as a primary "bulbar" type and that in (5) as indeterminate with respect to the general region of the neuraxis first affected by intraparenchymal inflammatory change

COMMENT

Since, unfortunately, blocks of the complete olfactory bulbs were available in less than half of the cases in this series, the negative findings as to involvement of these structures have correspondingly limited significance Yet they are in accord with the similar essentially

4 Luhan, J A Arch Neurol & Psychiat **37** 479, 1937

EXPLANATION OF FIGURES 9 TO 14

Fig 9—One plus or less involvement of the anterior gray matter of the cord Cresyl violet, $\times 67$

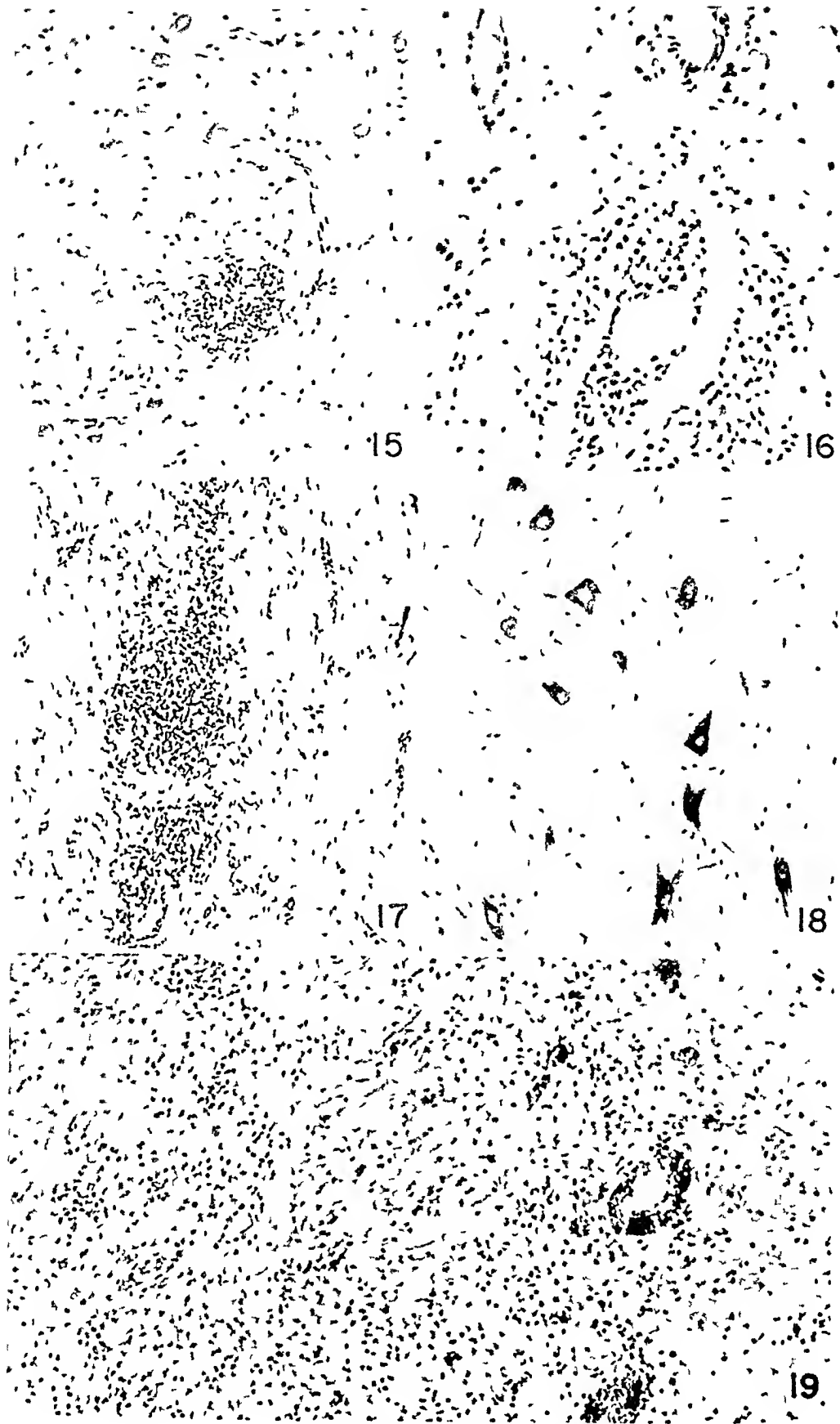
Fig 10—Three plus involvement of the anterior gray matter of the cord Cresyl violet, $\times 67$

Fig 11 (case 4)—Low magnification of the medulla oblongata which shows involvement graded $2\frac{1}{2}$ plus (perivascular infiltrations, 3 plus, other inflammatory changes, 2 plus) Cresyl violet, $\times 36$

Fig 12 (case 4)—Typical involvement of the substantia nigra Cresyl violet, $\times 70$

Fig 13 (case 6)—Dense inflammatory reaction in the region of the locus ceruleus of the pons Cresyl violet, $\times 68$

Fig 14 (case 6)—Neuronophagia within the facial nucleus Cresyl violet, $\times 87$



FIGURES 15 TO 19
(See legend on opposite page)

negative histologic observations of previous investigators,⁵ particularly Sabin,^{5d} who worked with material from the 1937 epidemic. On the other hand, experimental poliomyelitis induced by intranasal instillation of the virus is regularly found to be represented by perivascular and interstitial infiltrative lesions of the olfactory bulbs.⁶ Sabin and Ward⁷ were unable to demonstrate the presence of the virus of poliomyelitis in the olfactory bulbs in 6 cases of human poliomyelitis. Howe and Bodian² demonstrated that in at least a hundred cases of experimental poliomyelitis in which the virus was introduced by the nasal route, the olfactory bulbs were involved by perivascular cuffing and interstitial infiltration, in contrast to the absence of involvement of the olfactory bulbs in fifty-odd cases in which inoculation was by some portal other than the intranasal. These workers and others showed that in the chimpanzee the olfactory tract is an available route but not the so-called natural one. The macaque, on the other hand, is susceptible to inoculation by the olfactory channel and refractory to inoculation by the oral or the gastrointestinal route. In an able review of the problem of the significance of the nasal pathway, King⁸ stated, "It is possible that the relative importance of the nasal route in experimental poliomyelitis is purely an artificial condition, brought about by some degree of change in the virus and without necessary relation to the natural disease."

An interesting finding, confirming the work of a number of other investigators, is that the motor area, of all the cortical distribution,

5 (a) Horanyi-Hechst, B. *Deutsche Ztschr f Nervenhe* **137** 1, 1935 (b) Swan, C. *Australian J Exper Biol & M Sc* **17** 345, 1939 (c) Robertson, E G. *M J Australia* **1** 156, 1940 (d) Sabin, A B. *Am J Dis Child* **60** 1313, 1940.

6 Sabin, A B, and Olitsky, P K. *J A M A* **108** 21, 1937

7 Sabin, A B, and Ward, R. *J Exper Med* **73** 771, 1941

8 King, L S. *J A M A* **113** 1940, 1939

EXPLANATION OF FIGURES 15 TO 19

Fig 15 (case 4)—Compact or nodular focus in the dentate nucleus. Cresyl violet, $\times 105$

Fig 16 (case 2)—Perivascular microgliosis in the anterior paramedian white column of the first lumbar segment of the cord. Cresyl violet $\times 245$

Fig 17 (case 1)—Marked inflammatory reaction of the posterior gray column of the first sacral segment of the cord. (Higher magnification reveals the presence of numerous polymorphonuclear cells.) Cresyl violet, $\times 90$

Fig 18 (case 1)—Tissue from the anterior horn region of the same segment as the tissue shown in figure 17, revealing normal appearances. Cresyl violet, $\times 90$

Fig 19 (case 1)—Area of rarefaction in the anterior gray matter of the cervical part of the cord, diffuse type of cellular infiltration. Hematoxylin and eosin, $\times 125$

appears selectively vulnerable to the depredations of the virus. Involvement of the motor cortex in a case of human poliomyelitis was described in 1929 by Andre Thomas and L'Hermitte⁹, in that year Hurst¹⁰ published his report of comprehensive studies on the histologic aspects of experimental poliomyelitis, in which he pointed out that only in the motor area of all the cerebral cortex do the neurons succumb to the infection. Pette and his co-workers¹¹ substantiated Hurst's observation in their exhaustive studies of experimental poliomyelitis, published in 1932, they found that the inflammatory process was practically limited to the third and fifth layers within the motor area. Spielmeyer¹² in 1932 found the motor cortex implicated in 7 of 8 cases of human poliomyelitis and called attention to the selective involvement of the motor area in this disease. Kornyei¹³ (1933) reported that 8 human cases of poliomyelitis studied for distribution of the pathologic changes of the brain all showed lesions limited to the precentral area. Stiefler and Schenk¹⁴ described involvement of the motor area in 7 of 9 instances of human poliomyelitis in which the anterior central gyrus was examined. Horanyi-Hechst¹⁵ in 1935 found lesions in the precentral area in 19 of 24 human cases, with 3 showing lesions in the caudal part of the frontal agranular cortex also. Swan¹⁶ in a study of 8 cases of human poliomyelitis reported in 1939 found the area gigantopyramidalis of Brodmann involved in all, and with a single exception (lesions in the cornu ammonis) this was the only area of the cortex affected. Howe and Bodian² noted involvement of the motor cortex in 12 of 13 cases of human poliomyelitis, these lesions consisted of perivascular cuffing, neuronophagia and focal mesodermal glial infiltrations in all layers but especially in the layer of Betz cells. In 2 cases lesions were shown in area 6, in 4, in area 1 and in 2 there were infrequent lesions in the frontal granular cortex. That the virus may be recovered from the motor area in human poliomyelitis was demonstrated by Sabin and Ward.⁷

These reports together with the observations in the present study indicate that involvement of the motor area in the form particularly of interstitial mesodermal-glial infiltrations may be found in almost 90 per cent of cases of human poliomyelitis. This involvement, furthermore is selective in that either the motor area alone of all the cortex is involved or it is the most severely implicated of all the cytoarchitectural regions. In this study most of the sections of the motor area and of its

9 Andre Thomas and L'Hermitte, J. *Rev. neurol.* **1** 1242, 1929

10 Hurst, E. *J. Path. & Bact.* **32** 457, 1929

11 Pette, H., Denme, L., and Kornyei, St. *Deutsche Ztschr. f. Nervenheilk.* **128** 125, 1932

12 Spielmeyer, W. *Ztschr. f. d. ges. Neurol. u. Psychiat.* **142** 159, 1932

13 Kornyei, St. *Ztschr. f. d. ges. Neurol. u. Psychiat.* **146** 724, 1933

14 Stiefler, G. and Schenk, E. *Deutsche Ztschr. f. Nervenheilk.* **130** 68, 1933

vicinity were from the upper third (including that of the paracentral lobule) where the Betz cells are particularly numerous and easy to identify. Kornyei¹³ reported that the pathologic changes were the most pronounced in the upper third of the anterior central gyrus and were usually lacking in the lower third. Horanyi-Hechst¹⁴ found that the center for control of the leg was most severely involved as a rule. Swan¹⁵ did not find any consistent difference in the severity of the lesions in different areas of the motor cortex in his series; in 1 case the lower half of the gyrus was most heavily implicated. For the pathologist the practical significance of the relatively selective involvement of the gigantopyramidal motor area (FA₇ of von Economo and Koskinas) is that this peculiarity of distribution constitutes an aid to the presumptive identification of the disease. The Betz cell area, moreover, in practice is easily recognized.

The uniform occurrence of severe involvement of the reticular formation in the medulla in itself suggests that this may be an important factor in the fatal outcome of acute poliomyelitis.

There was no demyelination except in 2 cases in which there were foci of rarefaction of the ground substance of the gray matter of the cervical part of the cord as previously mentioned, but in this series of cases the disease was acute. In chronic cases some demyelination may be found in the cord (Peers¹⁵) and even cyst formation in the gray matter as in the case of a 26 year old woman who had been living in a respirator for six hundred and thirty-seven days (Baker¹⁶).

Whether or not the primary attack is on susceptible nerve cells and the inflammatory reaction is a secondary consequence of the chemical interaction of virus and ganglion cell has not been conclusively answered. Howe and Bodian² have adduced experimental evidence that living nerve cells are necessary for the production of typical lesions. They demonstrated absence of inflammatory reaction in the thalamus of an inoculated animal in which all nerve cells had previously disappeared from this structure after destruction of their axonal terminations in the cerebral cortex. Yet in poliomyelitis, whether experimental or human, the histologic changes show a disturbing lack of strict parallelism in topography and severity between the manifest disease or destruction of ganglion cells, the intraparenchymal mesodermal-glial reaction and the (mild) leptomeningeal infiltrations.

Although there is some evidence that the virus is disseminated within the neuraxis by axonal channels (for example, infection descending within the cord in 6 of 9 cases) it is not inconsistent with the hypothesis that the virus is not strictly neurotropic. For a virus to propagate only

15 Peers, J. H. *Am J Path* **19** 673 1943

16 Baker, A. B. *Journal-Lancet* **64** 224 1944

in nervous tissue and to seek immurement within the secluded neuraxis would mean its biologic suicide. There has been much experimental work within recent years to indicate that the virus may be found in the human alimentary tract, and the presumption follows that it may propagate there or at least outside the nervous system. Whether the hyperplasia of mesenteric and intestinal lymph nodes frequently observed in poliomyelitis bears some relation to the portal of entry of the virus was considered in this series. The most severe intestinal lymphatic hyperplasia (graded 3 to 4 plus) occurred in cases 1, 2 and 3, in which the infection was considered to be of primary "bulbar" type, although abdominal pain preceded the bulbar symptoms in case 1. The patient in case 3 (a man 20 years old) had no involvement of the olfactory bulbs, changes graded 3 plus in the medulla oblongata, $2\frac{1}{2}$ plus in the cervical part of the cord, 1 plus in the upper thoracic region of the cord and no lesions in the spinal cord below the tenth thoracic segment. In the remaining 3 cases there was moderate intestinal lymphoid hyperplasia, in 2 of these (11 and 12) the involvement was clinically of the spinal type in the beginning, with intercostal paralysis, and in the remaining case (5) the disease process was not easily classifiable with respect to initial neuraxial localization. In 1 case (9) of primary spinal poliomyelitis there was no significant hyperplasia of lymph nodes at necropsy.

SUMMARY

The pattern of distribution of the histologic lesions of poliomyelitis was studied in 13 fatal cases which predominantly were instances of the "bulbar" type of the disease. These cases occurred in the 1943 Chicago epidemic.

Involvement of the gigantocellular motor cortex was found in 12 of the 13 cases. This observation is in conformity with the findings of several previous investigators and argues for the presence of a "system factor" in poliomyelitis. It is also a clue to the identification of the virus so far as this may be ventured from the exhibition of pathologic changes in the central nervous system.

The olfactory route was probably not the important channel of inoculation in these cases.

Certain caprices of histologic reaction may be encountered in human poliomyelitis.

PRIMARY HYPERPARATHYROIDISM

A Report of Five Cases that Exemplify Special Features of this Disease (Infarction of a Parathyroid Adenoma, Oxyphil Adenoma)

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PRIMARY hyperparathyroidism is the condition that results from adenoma of the parathyroid glands. Characteristically in the majority of cases the associated clinical findings are hypercalcemia, hypophosphatemia, osteitis fibrosa cystica generalisata of varying degrees and/or renal parenchymal calcification and renal lithiasis. All of the associated abnormalities are effects produced by the excess of parathyroid hormone generated by the adenoma. Since the disease is due to a hypersecreting neoplasm of the parathyroid glands, it is quite properly designated by the term "primary hyperparathyroidism." This designation further serves to distinguish it from those states in which hypersecretion and hyperplasia of the parathyroid glandules result from, and are therefore secondary to, such prefatory conditions as chronic renal insufficiency.

Through the courtesy of Colonel J. E. Ash and Colonel B. Lucke,¹ I have been privileged to study the cases of parathyroid adenoma among the material collected at the Army Institute of Pathology in Washington, D. C. Because 5 of these cases exemplify many typical characteristics and a number of important special features of primary hyperparathyroidism they are reported in this communication.

REPORT OF CASES

CASE 1—Clinical Summary—A white man 21 years of age, a technical sergeant, was found on roentgenologic examination to have multiple osteolytic lesions involving the skull, the eleventh rib, the pelvis and the left femur. These skeletal lesions were asymptomatic. A segment of the eleventh rib was removed for diagnosis. Grossly the medulla of the rib was filled with soft brown tissue. This was surrounded by a thin cortex of brittle bone. Microscopically, the brown medullary tissue was made up of fibroblasts and multinucleated giant cells, some areas showed a tendency toward cyst formation. The pathologic diagnosis of osteitis fibrosa cystica was supported by chemical studies of the blood for calcium, phosphorus and phosphatase. At operation, Nov. 11, 1943, the neck was explored, and an adenoma of the left inferior parathyroid gland and also the normal right inferior parathyroid gland were removed.

From the Department of Anatomy of the Washington University School of Medicine, St. Louis, the Army Institute of Pathology, Washington, D. C., and the Division of Research in the Medical Sciences of the Lynn Clinic, Detroit.

Description of Specimens—The tumor from the region of the left inferior parathyroid gland was an irregular encapsulated body made up of soft brown tissue (fig 1). It measured 3.0 by 1.7 by 1.5 cm and weighed 3.9 Gm. The capsular surface was smooth and gray, the cut surface was brown and seemingly uniform in structure.

The tissue removed from the region of the right inferior parathyroid gland was a small brown body measuring 0.65 by 0.3 by 0.3 cm and weighing 0.05 Gm (fig 1).

Microscopically the tumor had a monotonously uniform structure (fig 2). The parenchyma consisted of closely packed epithelial cells arranged in irregular blocks that were separated by a delicate fibrous stroma containing many capillaries and sinusoids and that produced no particular type of pattern. No follicles or colloid deposits were seen. The parenchymal elements were nearly all dense cells,¹ clearly outlined, polyhedral of moderate size, with a finely granular, faintly azurophil cytoplasm and relatively large, centrally placed nuclei (fig 2). A perinuclear halo was present in a few cells which had the characteristics of small vesicular cells.

Pathologic Diagnosis—Parathyroid adenoma. The glandule removed from the right side was a normal parathyroid gland.

CASE 2—Clinical Summary—A white man 23 years of age had had scarlet fever and mumps at 14 and pneumonia at 17. Four years previously, in 1939, he had passed a small urinary calculus. In August 1943 he complained of pain in his back and of sluggishness, a tremor developed, and he became restless. Roentgen examination disclosed multiple parenchymal calcifications in both kidneys. The long bones, especially the upper end of the right femur, the pelvis and the skull were markedly decalcified and cystic.

During the last days of October 1943 the following laboratory data were assembled. The hemoglobin content of the blood was 12.2 Gm per hundred cubic centimeters. Red cells numbered 4,170,000 per cubic millimeter, white cells, 8,550, with polymorphonuclears 63 per cent, lymphocytes 34 per cent, eosinophils 2 per cent and monocytes 1 per cent. The serum calcium was 18.7 mg per hundred cubic centimeters, the phosphatase was 221 Bodansky units. The urea nitrogen of the blood amounted to 17.6 mg per hundred cubic centimeters. The urine contained albumin (3 plus) and many white blood cells.

Nov. 8, 1943 an adenoma was removed from the region of the left lower parathyroid gland. Following the operation, despite treatment, the blood calcium dropped rapidly to 7.2 mg in three days, but gradually rose thereafter to normal.

Description of Specimen—The tumor was an elongated ovoid mass measuring 2.2 cm in length by 1.0 cm in its greatest width and weighing 1.72 Gm. The surface was covered by a thin, rather opaque whitish capsule. On the cut surface the tumor was seen to be divided by a fibrous septum into two nodules, the neoplastic tissue was soft and presented a homogeneously pale, yellowish brown appearance.

Microscopically, the tumor was made up of irregular masses of closely packed epithelial cells, these parenchymal masses being outlined and separated by an extremely delicate vascular stroma (figs 3, 4 and 6). Although, as will be seen, there was some variation of the morphologic picture in different parts of the neoplasm, the most characteristic pattern was that illustrated in figure 3. The large majority of the parenchymal elements were typical vesicular cells¹, they

1 Norris, E. H. The Parenchymal Cytological Elements of the Human Parathyroid Glands, to be published.

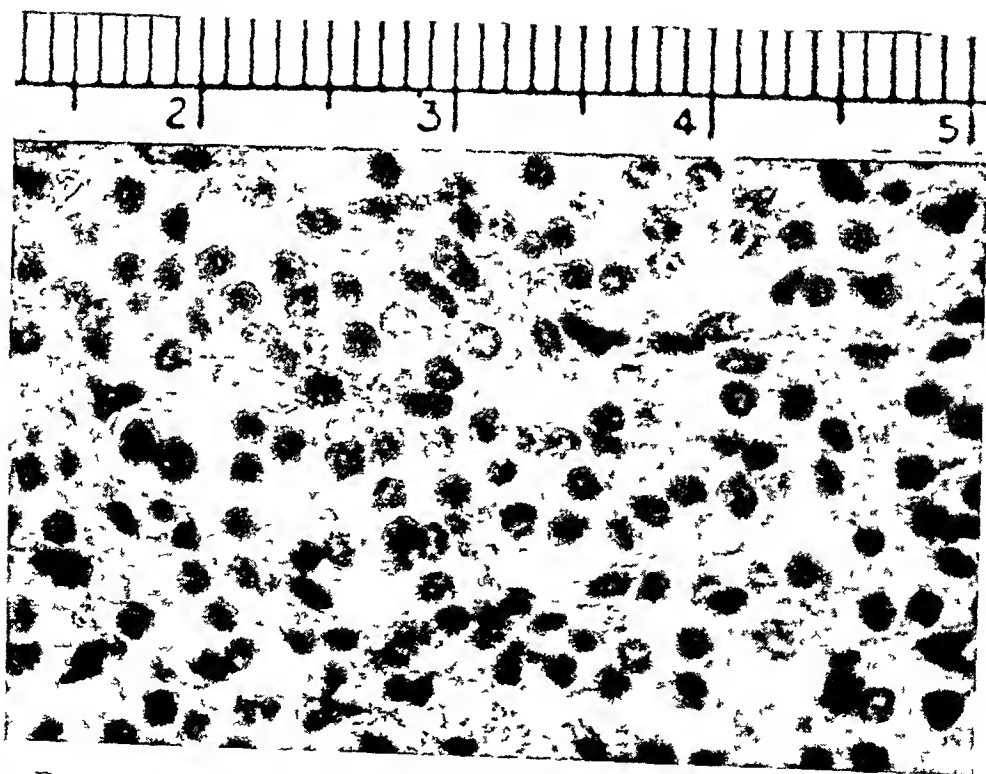


Fig 1—Photograph of the gross specimens removed in case 1. The small mass is the normal parathyroid gland and the larger is the adenoma.

Fig 2—Photomicrograph of a typical area from the adenoma of case 1. Note the monotonous character of the tumor. Nearly all of the cells are dense cells, only an occasional primordial or small vesicular cell is seen. $\times 700$

were outlined by distinct cell walls, were polyhedral and had a vacuolated non-stainable cytoplasm in which variable numbers of faintly azurophil granules were distributed. Such stainable cytoplasm as was present tended to accumulate at

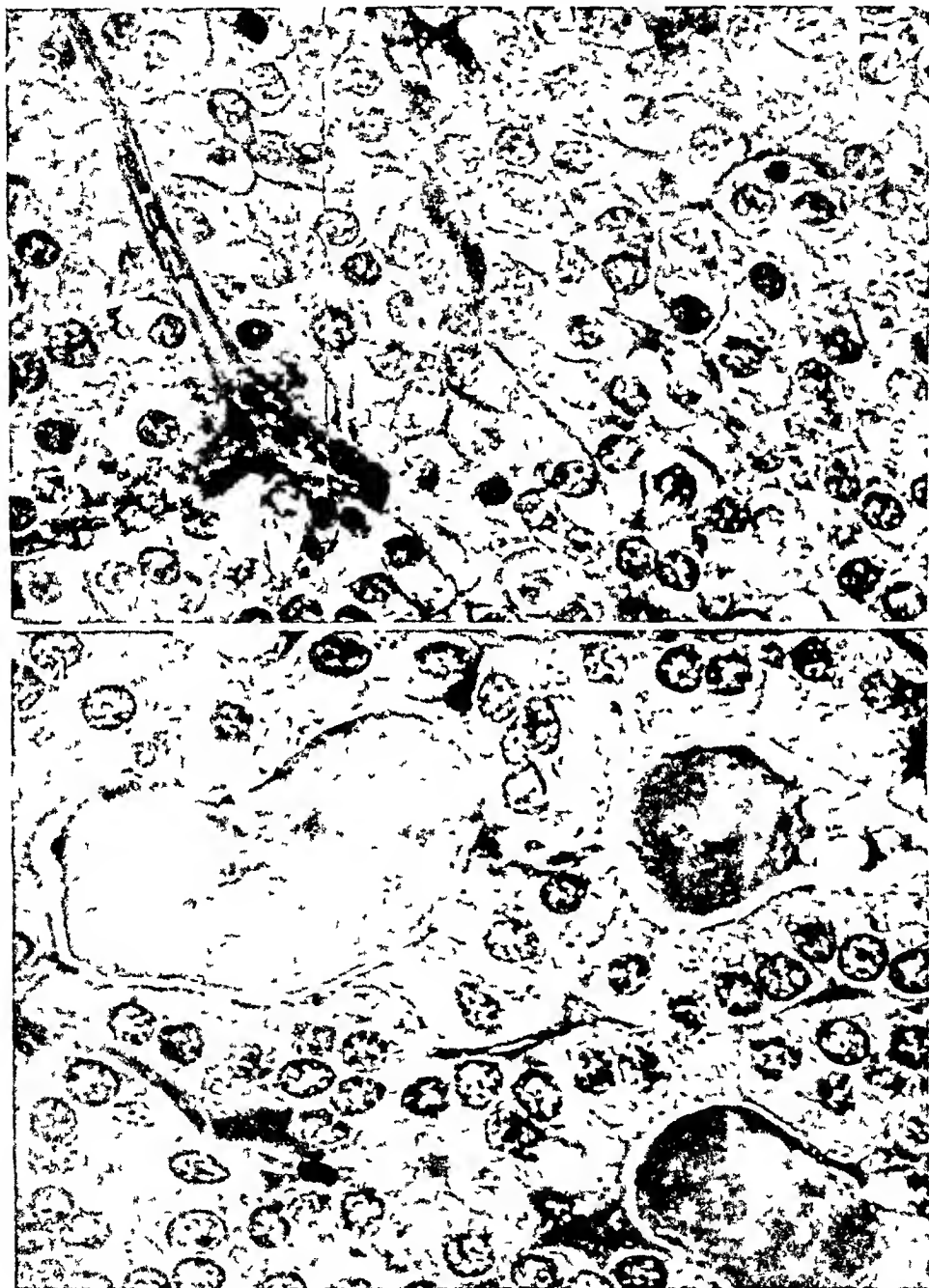


Fig. 3—Photomicrograph of an area typical of the greater part of the adenoma of case 2. Nearly all of the cells are vesicular cells, only an occasional primordial or dense cell is seen. $\times 700$

Fig. 4—Photomicrograph of a selected area in the adenoma of case 2 to illustrate an unusual structural pattern. Note the colloid-filled parathyroid follicles. Most of the cells are columnar dense cells, a few cells in the right upper corner may be called columnar vesicular cells. $\times 700$

the periphery of the cell, leaving a clear halo about the nucleus. The nuclei were relatively large, more or less round bodies surrounded by a thin but distinct nuclear membrane, and they contained a moderate amount of finely granular chromatin and prominent nucleoli (fig 3).

In some parts of the tumor the cells were arranged so as to form follicles (fig 4). The follicular lumens were more or less filled with eosinophilic hyaline colloid. Here and there some smaller droplets of colloid were found outside of follicles in the stroma near vascular elements. The cells of the follicular walls were columnar in form, and their rounded nuclei were located in the bases of the cells away from the lumen. Except that these particular cells had departed from the polyhedral form, their characteristics were typically those of dense or vesicular cells.

In still another part of the tumor, subjacent to the capsule, considerable numbers of large clear cells were found (figs 5 and 6). These cells had distinct walls and were polyhedral in form, their cytoplasm, which contained relatively few granules, was nearly nonstainable, and their nuclei were relatively small and pyknotic. As is apparent in figures 5 and 6, all manner of transitional forms were found between the vesicular cells and the large clear cells.¹

The capsule and the large stromal septums were composed of dense fibrous tissue. As illustrated in figure 7, nests of epithelial cells were found within this capsular tissue. Although such a picture suggests invasion, in the absence of other evidences of cancer, it is better interpreted as a marginal portion of the neoplastic parenchyma extending into the tissue spaces of the capsule.

Pathologic Diagnosis—Parathyroid adenoma

CASE 3—Clinical Summary—A white woman 48 years of age, a housewife, was first observed from July 7 to July 21, 1944. She complained of nervousness, fainting spells and slight convulsive attacks and called attention to a cervical swelling that had been present for some months. She stated that she had been well until June 25, 1944, when she suffered a severe convulsion at the onset of the present illness. Physical examination revealed a small mass in the neck at the level of the thyroid cartilage.

The following laboratory data were assembled. The hemoglobin content of the blood was 10 Gm per hundred cubic centimeters. Red cells numbered 3,740,000, white cells, 9,500, with lymphocytes 30 per cent, polymorphonuclears 68 per cent and monocytes 2 per cent. The blood creatinine amounted to 1.95 mg, the urea nitrogen, to 24.1 mg. The serum calcium was 5.0 mg per hundred cubic centimeters. The urine was normal. The rate of the basal metabolism was -7 per cent.

The patient was given large amounts of calcium gluconate with benefit. July 14, 1944 parathyroidectomy was performed.

Description of Specimen—The adenoma was removed together with a part of the lateral lobe of the thyroid gland, within which it was partially embedded. The adenoma was well encapsulated and measured 2.5 by 2.0 by 0.8 cm. On section the tumor was yellowish in color and almost caseous in consistency.

Microscopically, the major part of the tumor was made up of the shadow-like coagulated remains of epithelial cells that together constituted a necrotic mass. Under low power the cellular debris was seen arranged in closely related irregular blocks or strands—not unlike the usual pattern of a parathyroid adenoma (fig 8). The capsule was composed of rather dense fibrous tissue, between the layers of which were elongated islands of uninjured parathyroid tissue (fig 8). As illustrated in figure 9, these parenchymal islands contained a majority of clear cells.

with smaller numbers of vesicular and primordial cells. On the inner surface of the capsule and adjacent to the necrotic material there was some recent fibroblastic proliferation, some of these fibroblasts and a few more leukocytes had invaded the periphery of the necrotic mass. Considerable numbers of pigment-laden macrophages were seen in the looser outer layer of the capsule.

Pathologic Diagnosis—Parathyroid adenoma with recent infarction



Fig 5—Photomicrograph of a subcapsular region in the adenoma of case 2. The majority of the cells in this area are large and small clear cells, with some vesicular and a few dense cells in the left upper corner. $\times 150$

Fig 6—Photomicrograph of a small field taken from near the center of the region pictured in figure 5. Large and small clear cells are in the majority, some vesicular cells are noted in the right upper corner. $\times 700$

CASE 4—*Clinical Summary*—A white man 27 years of age, a sergeant in a supply and service company, came under observation, May 3, 1944, because of a tumor in the right side of the mandible which had been noticeable for about six months. The mandibular lesion, excised May 6, was made up of brownish

tissue containing spicules of bone. Microscopically, the tissue consisted of loose cellular connective tissue in which numerous multinucleated giant cells were dispersed. A diagnosis of "benign giant cell tumor of the mandible" was made.

About a year later the patient was admitted to the urologic service because of pain in the region of the right kidney. He stated that on the average at least one renal stone had been passed every year since 1927 (eighteen years). The last stone was passed in May 1944, and since then there had been intermittent pain in the region of the left kidney. Roentgenologic examination revealed dysfunction of both kidneys with diffuse calcification.

The following laboratory data were assembled. The serum calcium amounted to 14.0 mg and the phosphorus to 1.8 mg per hundred cubic centimeters, the phosphatase value was 8.0 Bodansky units. A twenty-four hour specimen of the urine, 2670 cc, contained 19.5 mg of calcium per hundred cubic centimeters.

May 17, 1945 an adenoma was removed from the region of the left upper parathyroid gland. The postoperative course was excellent. The serum calcium dropped quickly to 10 mg per hundred cubic centimeters, and the calcium content of the urine became normal.

Description of Specimen—The tumor was a rather soft, well encapsulated mass that measured 2.6 by 1.1 by 0.6 cm, the tissue had a tannish gray color.

Microscopically, the tumor had a uniform structure. With one exception its constitution was similar to that of the neoplasm observed in case 1, in the parenchyma dense and vesicular cells were about equally numerous.

Pathologic Diagnosis—Parathyroid adenoma.

CASE 5—Clinical Summary—A white woman 23 years of age, a Women's Army Corps clerk, noticed a lump on the right side of her neck about June 1, 1945. It caused no trouble except when she wore a tight collar or when she tried to swallow a large mouthful of food or fluid. No other symptoms were noted except that the last two menstrual cycles had changed from "28—4 day type" to a "28—1 or 2 day type."

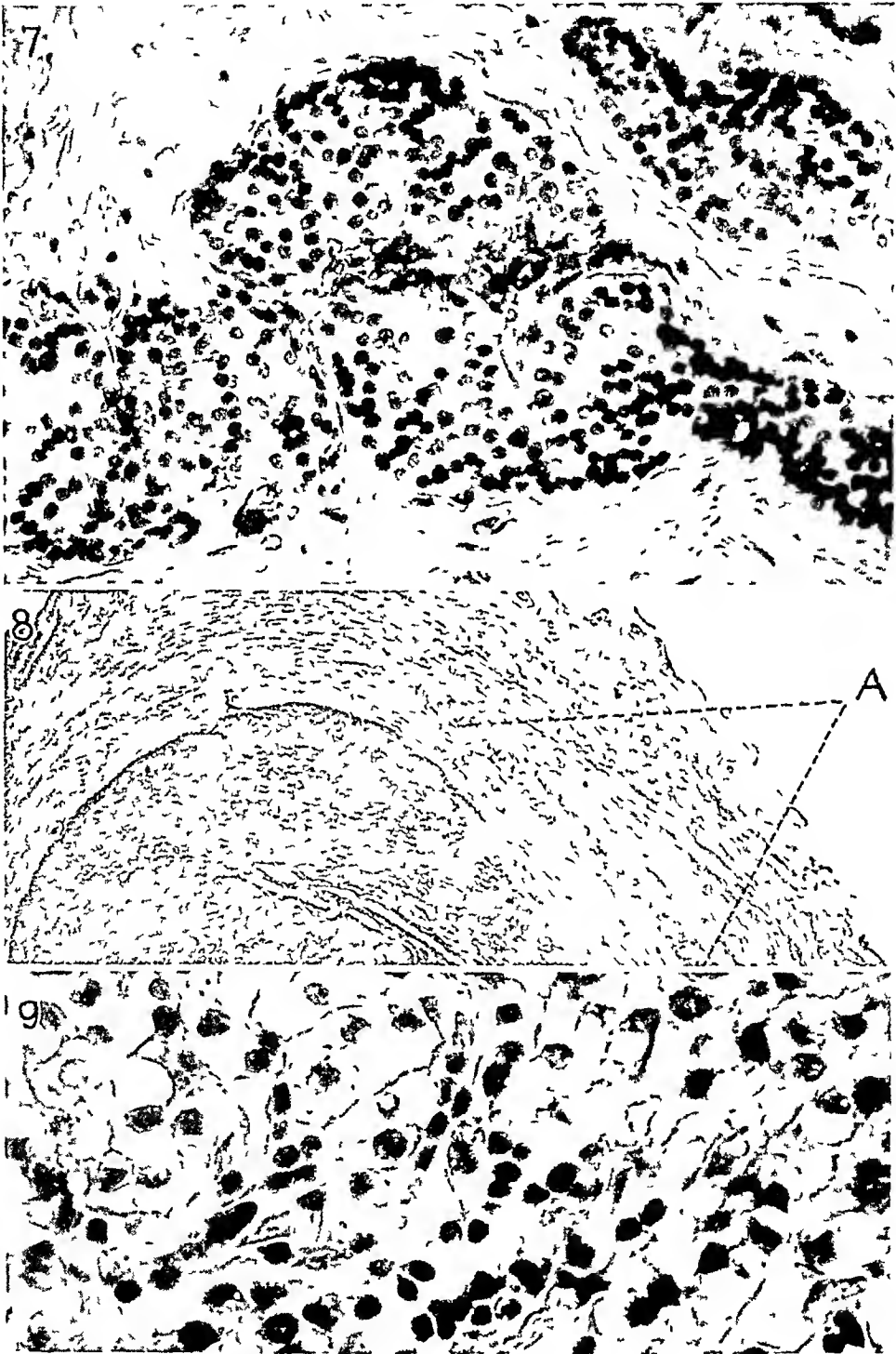
But for the swelling in the right side of the neck the physical examination showed no abnormality. In the lower lateral portion of the neck just anterior to the anterior border of the right sternocleidomastoid muscle was a small round smooth mass, approximately 4 cm in diameter. The mass seemed to be attached to the thyroid gland and moved with the thyroid gland on swallowing.

Roentgenographically, the chest was normal, and there was no evidence of tracheal deviation or of substernal thyroid gland. Roentgenograms of the skeletal system revealed no decalcification or cystic changes.

The laboratory data included the following observations. The blood calcium amounted to 12.3 mg per hundred cubic centimeters June 12 and 12.8 mg June 28. The basal metabolic rate was —13 per cent June 14 and —10 per cent July 9. Serologic tests revealed no syphilis. The urine, the red blood cell count, the white blood cell count and the hemoglobin content were normal.

July 12 (four weeks after the cervical mass was first noticed by the patient) she was operated on. An adenomatous tumor occupied all of the right lobe of the thyroid gland except the upper pole, therefore, subtotal resection of the right lobe of the thyroid gland was done.

On the first day after operation the patient complained of tingling and numbness of the fingers and the toes and showed hyperirritability of the facial nerves, otherwise the postoperative course was normal. August 13 the blood calcium was 10.2 mg and the blood phosphorus 3.5 mg per hundred cubic centimeters. The blood phosphatase value was 9.1 units.



FIGURES 7, 8 AND 9
(See legend on opposite page.)

Description of Specimen—The tumor consisted of a well encapsulated nodule 3.5 cm in diameter, partially surrounded by thyroid tissue

Microscopically, the tumor had a uniform structure (fig 10). The parenchyma was composed of sheets of closely packed epithelial cells. The stromal elements and the vascular channels were delicate and scattered in such a way as to divide the epithelial tissue into blocks and masses of irregular form. An occasional droplet of extrafollicular colloid was noted, but there were no follicles.

Nearly all of the cells were of one type—relatively large, with distinct walls. As compared with typical primordial or dense cells, many in this tumor had preserved their polygonal form, but there was a definite tendency toward reduction in the number of angles and replacement of straight sides by smoothly curving outlines. In other words, these cells approached forms that were more

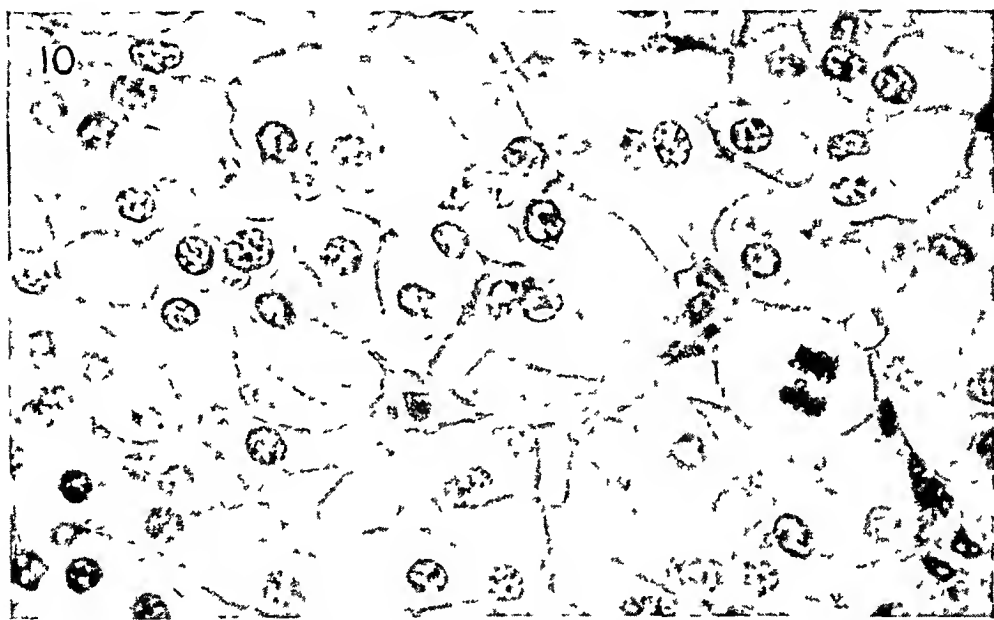


Fig 10—Photomicrograph of a typical area from the adenoma of case 5. Practically all of the cells in this tumor are large pale oxyphil cells. $\times 661$

nearly quadrangular than polyhedral, with gentle curves replacing sharp angles. The cytoplasm was made up of evenly distributed fine eosinophilic granules—in some cells, a bit more coarse. An occasional mitosis was seen. Although in

EXPLANATION OF FIGURES 7, 8 AND 9

Fig 7—Photomicrograph of nests of parenchymal cells that have extended from the adenoma into the tissue spaces of the dense connective tissue that makes up the capsule of the adenoma. $\times 354$

Fig 8—Photomicrograph of a section from the adenoma of case 3. Note the complete necrosis of the main body of the adenoma, and the surrounding uninjured capsule. The leaders marked *A* indicate two wedge-shaped islands of uninjured parathyroid parenchyma lying between layers of the capsule. $\times 19$

Fig 9—Photomicrograph of an area from one of the capsular islands shown in figure 8. These parenchymal cells are apparently uninjured and are good examples of vesicular and clear cells. $\times 661$

certain areas a few vesicular and dense cells were present, the majority of the cells of this tumor were morphologically like the large pale oxyphil cells of normal glandules (fig 10)

Pathologic Diagnosis—Parathyroid adenoma

COMMENT

De Santi² in 1900 and Benjamins³ in 1902 are often credited with having been the first to describe primary tumors of the parathyroid glands, however, in the light of present knowledge their descriptions are not convincing. De Santi did not report on the histologic aspects of the tumor which he observed. The tumor Benjamin described was as large as a child's head¹. And despite the fact that both these tumors were large, neither author recorded skeletal changes. Other observers of the same period (Kocher,⁴ Langhans⁵ and de Quervain⁶) recorded cases of cervical tumor that may have arisen from the parathyroid glands, but again the histologic evidence of origin is not clear. During the first three decades of the present century there appeared scattered reports of parathyroid tumors found at autopsy. Probably the earliest record of a case that may be accepted today as one of parathyroid adenoma was that presented by Erdheim⁷ in 1903. A year later Askanazy⁸ was the first to call attention to the association of a parathyroid tumor and skeletal lesions. Thereafter the association of an enlarged parathyroid gland and osteitis fibrosa cystica generalisata was recognized, but it was not until the first time that an enlarged parathyroid gland was surgically removed, by Mandl⁹ in 1925, that widespread practical interest was stimulated. Since 1925 there has been a rapidly growing list of case reports, mostly from surgical clinics. Norris¹⁰ in a recent survey of the literature showed that more than 300 cases of parathyroid adenoma have been reported. However, because no one investigator has had the opportunity to study more than a few cases, it is not surprising that fundamental and practical knowledge of primary hyperparathyroidism has developed gradually.

The 5 cases reported in this paper exemplify features to which special attention may be called. Certain of these features are of practical clinical and pathologic interest. In addition, it must not be for-

2 De Santi. Internat Centralbl f Laryng u Rhin **16** 546, 1900

3 Benjamins, C. E. Beitr z path Anat u z allg Path **31** 143, 1902

4 Kocher, T. Virchows Arch f path Anat **155** 532, 1899

5 Langhans, T. Virchows Arch f path Anat **189** 69, 1907

6 DeQuervain, F. Deutsche Ztschr f Chir **100** 324, 1909

7 Erdheim, J. Beitr z path Anat u z allg Path **33** 158, 1903

8 Askanazy, M. Arb a d path Inst zu Tubingen **4** 398, 1904

9 Mandl, F. Wien klin Wchnschr **38** 1343, 1925

10 Norris, E. H. The Parathyroid Adenoma, Surg, Gynec & Obst., to be published

gotten that the careful study of such cases as are reported in this communication offers another important potentiality and much needed facility, the possibility of analyzing the functions of the different kinds of parathyroid cells in correlation with their histologic structure and chemical activity in pathologic states promises an advance toward the understanding of their normal physiologic and metabolic functions

Case 1, case 2 and case 4 present features that may be compared and contrasted. In each the adenoma was single and of moderate size and was associated with hypercalcemia and the characteristic lesions of osteitis fibrosa cystica generalisata. In addition, case 2 and case 4 were complicated by renal lithiasis and calcification. There can be no doubt that dense cells are secretorally active, for the adenoma of case 1 was composed almost entirely of cells of this type. In the tissue in case 4 dense cells and vesicular cells were about equally numerous. In contrast, the adenoma of case 2 has a great preponderance of vesicular cells, and the serum calcium was found at a high level here too, however, there is no room for doubting that vesicular cells are secretorally active. Nevertheless, two questions are posed. Elsewhere¹ I have expressed my opinion that vesicular cells are functionally more active than dense cells. Can this opinion be supported by the regular finding of higher serum calcium levels in those cases in which the adenoma contains a preponderance of vesicular cells? Is it in those cases of primary hyperparathyroidism in which the higher levels of serum calcium are observed that renal lithiasis and calcification tend to occur? Although it seems likely that ultimately both queries may be answered in the affirmative, the data recorded for these 5 cases are insufficient and the information to be found in the literature is inadequate to establish the concepts. To increase their value, future case reports should contain a careful and complete record of the information needed to enable exhaustive analysis of groups of reported cases.

In 1935 Castleman and Mallory¹¹ presented an excellent pathologic study of the parathyroid gland as observed in cases of hyperparathyroidism and they differentially delineated the features of adenoma and hyperplastic glands. These authors stated

the histological picture of the hyperplastic gland, at least of the more common wasserhelle type, is so characteristic, so different from anything we have seen in the cases of single tumor formation that we believe a diagnosis of hyperplasia should be possible as a rule from the histological examination of a single gland, even from a frozen section during an operation. The uniform, giant sized clear cells, the acinar arrangement, the basal orientation of the nuclei form a readily recognizable picture.

In general my experience agrees with the statement quoted. However, as in cases 2, 3 and 4, I have seen sufficient exceptions to the rule to

¹¹ Castleman, B, and Mallory, T B. *Am J Path* **11** 1, 1935

caution against too broad generalization. The variety of cell types and structural patterns illustrated in figures 3, 4, 5, 6 and 7 and all found in the adenoma of case 2 are considerable.

The striking absence of oxyphil cells in the adenoma in cases 1, 2 and 4 is a point worthy of attention. This observation accords with the findings in the majority of the cases of adenoma that have been described. I interpret the absence of oxyphil cells to signify two things. First, if oxyphil cells were present in the gland prior to the development of the adenoma, they must have reverted to the cell type from which they were derived. Second, oxyphil cells are probably concerned with some other function than that carried on by the primordial, vesicular, clear, dense, and dark cells. The adenoma of case 5 was true oxyphil adenoma, and it is important to note that the only clinical symptom was a cervical swelling, the patient had no skeletal or renal symptoms or signs, and there was little elevation of the serum calcium. A nearly identical case was reported by Cope¹² in 1944, the patient had a nodule in one side of the thyroid gland which turned out to be an oxyphil adenoma. Cope stated

A parathyroid tumor was not suspected preoperatively and the chemical studies necessary to exclude hyperfunction were, therefore, not made. It is probable, however, since there was no clinical tetany after removal of the tumor, that hyperfunction did not exist. This belief is strengthened by Castleman's opinion that the presence of pale oxyphil cells in large numbers in an adenoma indicate nonhyperfunction.

In 1938 McQuillan¹³ reported a similar case of oxyphil adenoma, the patient suffered only from pressure symptoms (dysphagia) due to a cervical tumor, and the bones and the blood chemistry were found to be normal.

The prominence of oxyphil cells in the normal parathyroid glands of adults being kept in mind, the inconspicuousness of these cells in most cases of parathyroid adenoma and the picture presented in cases such as these 3 should guide in determining the function of oxyphil cells.

In the adenoma of case 2 the presence of areas of clear cells bespeaks a high degree of functional activity for the tumor. This tumor provides a fortunate situation for observations on the genetic relationship of primordial, vesicular and clear cells, all manner of transitional stages between these three cell types are present.

Apparently the histologic structural pattern of parathyroid adenoma is subject to considerable variation. Of these variations certainly the monotonous uniformity found in case 1 is the simplest. In the tumor of case 2 the colloid-filled follicles and extrafollicular droplets of colloid add variety to the structural arrangement, I am of the opinion that such

12 Cope, O. *Surgery* **16** 273, 1944.

13 McQuillan, A. S. *Ann Surg* **108** 464, 1938.

histologic variations are largely accidental and that no important significance can be attached to them. Similar findings are observed from time to time in normal glandules.

Case 3 illustrates a most unusual bit of parathyroid pathology. There is nearly a total infarction of the adenoma. The infarction is recent, and the histologic changes (necrosis and cellular reaction in the capsule of the tumor) are such in character and degree as would correspond to the patient's clinical history of twenty days' duration. From the specimen removed at operation it is not possible to determine the cause of the infarction. However, the clinical effect of the infarction was dramatic, it produced sudden severe hypoparathyroidism, associated with spasms and convulsions and with an alarming degree of hypocalcemia. Indeed, the spontaneous symptoms of hypoparathyroidism with which this patient suffered were similar to those of the hypoparathyroidism which may follow surgical removal of a parathyroid adenoma. This case is interesting also from the point of view of interpreting the function of the parenchymal cytologic elements of the gland. Although there were several thin layers of uninjured parenchyma in the capsule of the adenoma, and although these layers were made up of vesicular and clear cells, they were not functionally adequate to prevent the development of a hypoparathyroid state. One may conclude that at least within certain limits there is a quantitative relationship between the amount of parathyroid parenchyma and the secretory activity of the glandules, and the clinical state of the patient.

SUMMARY

In 5 cases of parathyroid adenoma the tumor was single and of moderate size.

In 3 of the cases the adenoma was associated with hypercalcemia, hypophosphatemia and the skeletal changes of osteitis fibrosa cystica generalisata. In 2 of these 3 cases there were manifestations of renal calcifications and lithiasis.

In 1 case there was nearly complete infarction of the parathyroid adenoma. The patient came under clinical observation in a severe state of hypoparathyroidism (hypocalcemia and convulsions).

In 1 case the tumor was a true oxyphil adenoma. This is a rare neoplasm. It was associated with no skeletal or renal lesions.

Although more than 300 cases of adenoma of the parathyroid glands have been reported in the literature, knowledge of primary hyperparathyroidism is still incomplete.

Primary hyperparathyroidism provides a favorable situation for analyzing the function of the different kinds of parathyroid cells in an effort to correlate their histologic structure and chemical activity, such study promises an advance toward understanding the normal physiologic and metabolic functions of these cell types.

FUNCTIONING OF THE FETAL KIDNEY AS REFLECTED BY STILLBORN INFANTS WITH HYDROURETER AND HYDRONEPHROSIS

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AND
E T BELL, M D
MINNEAPOLIS

JUDGING from observations in fetal rats,¹ we believe that the rate of secretion of fetal urine is much more rapid than formerly supposed.² The rate may be accelerated experimentally by injecting urea under the skin of the fetus or by ligating the renal pedicles of the mother.³

A review of literature for evidence that the human kidney functions before birth led to the realization that there are surprisingly few records of stillborn infants who showed hydronephrosis or urine in the bladder or both. In fact, the only records found were those of 6 infants observed by Dohrn.⁴ The quantity of urine in the bladder ranged

From the Departments of Anatomy and Pathology, University of Minnesota

1 Wells, L J Anat Rec 94 504, 1946

2 (a) Gersh, I Contrib Embryol 26 33, 1937 (b) Windle, W F Physiology of the Fetus, Philadelphia, W B Saunders Company, 1940 (c) Preyer, W Specielle Physiologie des Embryo, Leipzig, Grieben, 1885

3 Wells, L J Proc Soc Exper Biol & Med 62 287, 1946

4 (a) Hinman, F The Principles and Practice of Urology, Philadelphia, W B Saunders Company, 1935, vol 1 (b) Parmelee, A H The Newborn Child, in Curtis, A H Obstetrics and Gynecology, Philadelphia, W B Saunders Company, 1933, vol 1 (c) Bell, E T A Textbook of Pathology, Philadelphia, Lea and Febiger, 1944 (d) Needham, J Chemical Embryology, London, Cambridge University Press, vol 3, pp 1255-2019, 1931, (e) Gruber, G B Missbildungen der Harnorgane, in Schwalbe, E Die Morphologie der Missbildungen des Menschen und der Tiere, Jena, G Fischer, 1927, pp 157-374 (f) Ahlfeld, F Arch f Gynäk 14 276, 1879 (g) Virchow, R Gesammelte Abhandlungen zur wissenschaftlichen Medizin, Hamm, G Grote, 1862 (h) von Bischoff, T L W Entwicklungsgeschichte der Säugethiere und des Menschen, Leipzig, Leopold Boss, 1842 (i) English, J Arch f Kinderh 2 98, 1881 (j) Dohrn Monatsehr f Geburtshunde u Frauenkrankheiten 29 105, 1867 (k) Cameron, G, and Chambers, R Am J Physiol 123 482, 1938 (l) Makepeace, A W, Fremont-Smith, F, Dailey, M E, and Carroll, M P Surg, Gynec & Obst 53 635, 1931 (m) Guthmann, H, and May, W Monatsehr f Geburtsh u Gynäk 91 306, 1932 (n) Litzmann Fotalleben, in Wagner, R Handwörterbuch der Physiologie, mit Rücksicht auf physiologische Pathologie, Braunschweig, F Vieweg u Sohn, 1842-1853, vol 3, pt 1, p 91 (o) Hecker, C Virchows Arch f path Anat 11 217, 1857 Gersh^{2a} Windle^{2b} Preyer^{2c}

from 4 to 105 cc, and only the infant having the most urine showed hydronephrosis (*Ureteren und Nierenbecken zu Cysten entartet* [ureters and renal pelves degenerated to cysts])⁵

In searching the necropsy files of the department of pathology of the University of Minnesota for such records, we centered attention on those of the 52 stillborn infants which showed spina bifida. These infants would be expected to exhibit a high incidence of anomalies. The finding of a single one with dilatation of the urinary passages would constitute evidence that fluid had been secreted previous to the interruption of the placental circulation. Four were found (table).

Considering the anatomic causes of the dilatations and the steps in embryonic development leading to the anomalous conditions, we find it obvious that in the first infant the obstruction of the passages was due to the blind ending of the ureters associated with absence of

Stillborn Infants with Hydroureter and Hydronephrosis¹

| Infant | Sex | Crown Heel Measure- ment, Cm | Age, Lunar Months * | Anomalies of the Urinary Passages | | |
|--------|-----|---------------------------------------|---------------------------|--|---|--------------------------------------|
| | | | | Dilatations | Obstructions | Other Anomalies |
| 1 | F | | 7† | Bilateral hydroureter (diameter of ureters, 1 to 2 cm) | Blind ending of ureters | Absence of bladder and urethra |
| 2 | F | 53 | 10 | Left hydroureter | Thin membrane closing left ure- teral orifice | Horseshoe kidney |
| 3 | M | 36 | 7 | Bilateral hydrone- phrosis and hydrou- reter (slight) | None recorded | None recorded |
| 4 | M | 45 | 9 | Left hydronephrosis and hydroureter | None recorded | None recorded |

* The ages of the second, third and fourth infants were estimated by interpolation from Richard E. Scammon's table of ages based on crown heel measurements (unpublished)

† The age of this infant was estimated from the menstrual history of the mother

the bladder and urethra. The presence of ureters shows that early in development the wolffian ducts had entered the cloaca, since in embryos of chicks agenesis of the ureter may be produced experimentally by preventing the wolffian duct from reaching its normal destination⁶. The presence of kidneys indicates that the ureteric buds, acting as organizers, had caused the metanephric blastemas to differentiate into secretory tubules, because in human embryos nonunion of bud and blastema results in agenesis of the kidney⁷. Subsequent to these steps there had been arrested development of the cloacal region. This is

5 We did not attempt an exhaustive survey of the literature, and we excluded from consideration numerous records in which it was impossible to determine whether the infants were stillborn (Englisch⁴¹, Dohrn⁴², Makepeace and co-workers⁴¹)

6 Boyden, E. A. *Anat. Rec.* **52**: 325, 1932

7 Gruenwald, P. *Anat. Rec.* **75**: 237, 1939. Boyden⁶

shown by the fact that at autopsy the infant lacked bladder, urethra, genitalia, anus and rectum

In the second infant the anatomic cause of the dilatation was the thin membrane which obstructed the end of the left ureter. Undoubtedly this membrane represents anomalous persistence of the ureteral septum, described by Chwalla⁸. In normal embryos this septum completely seals off the end of the ureter for a considerable period. It appears at the stage of development in which the ureter has just established its direct opening into the urogenital sinus, and it usually persists until about the 24 mm stage. Although in this infant there was no other anomaly which reflected a retardation of development subsequent to the 24 mm stage, the presence of a horseshoe kidney pointed to such retardation previous to this stage. A causative factor in the development of horseshoe kidney is a delayed passing of the kidneys through the crotch of the umbilical vessels, and the critical moment for fusion of the kidneys is during or near the 10 mm stage⁹.

In the third and fourth infants there may have been membranes closing or partially closing the ends of the ureters that were not observed at autopsy. That a defective nerve supply of the bladder did not cause the hydronephrosis, as it may in cases of anencephaly,^{4c} is suggested by the fact that in the third infant the bladder was listed as normal and that in the fourth the dilatation was unilateral.

These observations confirm those of Dohrn,¹¹ and they provide evidence, as convincing as any thus far recorded, that in the human fetus the kidney secretes fluid. Cameron and Chambers^{4b} found that as early as three and one-half months after conception the fetal kidney is able to concentrate vital dyes. Also, analysis of the fluids of pregnancy suggests that fetal urine enters the amniotic sac, especially during the last half of gestation^{41,m}. Furthermore, in some cases of birth by breech presentation, it has been noted that previous to delivery of the head a small amount of liquid emerged from the urethral orifice¹⁰. Contrary to one point of view,¹¹ it would seem that the several observations constitute ample evidence for concluding that the human kidney functions during the prenatal period, especially when these observations are considered in the light of the rapid secretion of urine by the kidney of the fetal rat¹².

8 Chwalla, R. *Ztschr f Anat u Entwicklungsgesch* **83** 615, 1927

9 Boyden, E. A. *Anat Rec* **51** 187, 1931. Boyden^c

10 Litzmann⁴ⁿ. Hecker^{4o}

11 Gruenwald, P., and Popper, H. *J Urol* **43** 452, 1940

12 Footnote 3

EXPERIMENTAL CHOLESTEROL ARTERIOSCLEROSIS

Changes Produced in Skeletal Muscle

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EXPERIMENTAL arteriosclerosis may or may not be identical with, or related to, human arteriosclerosis. Nevertheless, experimental arteriosclerosis is an excellent means of studying vascular changes and their sequelae and of revealing potential reactions of the vascular system.

The present report deals with experimental cholesterol arteriosclerosis. The effect of feeding egg yolk and other cholesterol-containing foods or pure cholesterol to herbivores has been amply studied. Extensive reviews have been contributed by Duff¹ and Hueper².

The greater part of the arterial tree becomes involved, as well as many other tissues and organs. Yet, until recently it has seemed impossible to produce changes in vessels of the central nervous system, in peripheral nerves and in skeletal muscle. I³ have succeeded in producing vascular changes in the cerebral vessels of 5 of 17 rabbits, in addition to the regular changes in the choroid plexus which have been described previously. In several rabbits the peripheral nerves were also affected. The skeletal muscle was examined in 17 rabbits, 7 guinea pigs and 2 golden hamsters, and in all of these animals with the exception of 2 guinea pigs which died shortly after the beginning of the experiments, muscular changes were evident. The description of the lesions occurring in skeletal muscle follows.

METHODS

The animals were given a diet of milk, powdered yolk and yolk cake. The yolk cake was baked from a dough of 4 volumes (approximately) of yolk powder, 1 volume of flour, yeast, water and a little salt. To this diet some hay was added from time to time. A number of rabbits received, either from the beginning of the experiment or later on, a daily dose of 0.3 Gm of pure cholesterol in capsules. No oil was given to facilitate the absorption of the cholesterol because the fat contained in the milk and in the yolk sufficed for this purpose.

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This work was supported by a grant from the Daland Fund of the American Philosophical Society.

1 Duff, G. L. Arch Path 20: 81 and 259, 1935.

2 Hueper, W. C. Arch Path 38: 161, 245 and 350, 1944, 39: 51, 117 and 187, 1945.

3 Altschul, R. J. Neuropath & Exper Neurol, to be published.

The animals died or were killed at certain intervals. Skeletal muscle was excised from different regions, mainly from the thigh, the dorsal musculature, the diaphragm, the forelegs, the masseters and the tongue. It was fixed twenty-four to forty-eight hours in 4 per cent formaldehyde solution, then trimmed, dehydrated and embedded in paraffin. Sections were stained by the following methods: hematoxylin-eosin, cresyl violet, Mallory's for connective tissue, von Kossa's for calcium, Gomori's for iron.

RESULTS

The results varied greatly from one animal to another and among muscles of different regions. Two different types of lesions were observed, namely, those of the muscle fibers proper and those of the blood vessels in the interstitial tissue. In 1 case alterations were found inside a muscle spindle. Nerves of the muscles were also affected, but these lesions have been reported elsewhere.³

Changes in the Muscle Fiber Proper —At least three types of changes were observed. The first and most severe was waxy (hyaline) degeneration of the sarcoplasm with disappearance of fibrillae and muscle nuclei. Frequently, this change affected only parts of the fiber. In these cases, the waxy part of the degenerated fiber was round and wider than normal, forming a kind of homogeneous eosinophilic mass intercalated in the fiber. When the adjacent portions of the fiber were affected, they were abnormally thin, were basophilic and showed nuclear proliferation.

In the second type of change the damage was less severe. Here the sarcoplasm turned distinctly basophilic, lost its cross striation, became granular and broke into particles. The Kossa reaction for calcium salts was distinctly positive, while the non reaction of Gomori revealed only traces of iron. I could not determine whether the presence of calcium salts was responsible for the basophilia of the fibers or merely accompanied it. The nuclei of the muscle did not disappear. On the contrary, they proliferated to a lesser or greater degree. They were frequently surrounded by some basophilic substance and separated from one another. In this event they may be regarded as muscle corpuscles being liberated from the syncytium of a single muscle fiber.

The separation of the muscle corpuscles from the degenerating fiber corresponds possibly to what Pfuhl⁴ has termed dissociating degeneration, though in his opinion it occurs only in the first thirty-six hours after acute injury of the muscle fibers, and all the liberated muscle corpuscles degenerate soon. Pfuhl's statement is based on experiments of short-lasting injury (trypan blue injected into and around skeletal muscle).

In some cases the proliferating nuclei accumulated in piles on the periphery of the fiber, abandoning, as it were, the degenerated sarcoplasm and fibrillae (fig. 1). This anuclear fiber material became still more basophilic and calcified.

4 Pfuhl, W. Ztschr. f. mikr.-anat. Forsch. **41** 569, 1937.

In a few cases the proliferation of nuclei was extreme, hundreds of nuclei appeared in greatly enlarged fiber portions where before the onset of the change two or three nuclei may have occurred (fig 2) Under such circumstances it was not surprising to see some degenerating nuclei, for, as Bloom⁵ stated " in most rapidly growing tissues, there is an occasional degenerating cell " Moreover, in the present case one was dealing with a pathologic condition

In the third type of change the damage was even less severe The fiber was very thin, the sarcoplasm was basophilic and the nuclei, after migrating to the center of the fiber, proliferated and formed longitudinal nuclear rows There were differences in degree, leading gradually to the normal fiber

None of the changes thus far described are specific for experiments with cholesterol feeding All of them may occur in traumatic injuries, some of them in nutritional myodegeneration (Goettsch and Pappenheimer⁶, Chor and Davenport⁷), in avitaminosis E (Pappenheimer⁸), in toxemias and infectious diseases (Zenker's well known waxy degeneration in typhoid), while the thinning of fibers with nuclear proliferation occurs regularly in denervation Therefore, it is logical to attribute these changes not to arteriosclerosis or to cholesteremia directly but, until the contrary is shown, to the serious changes affecting the liver and to the lesser changes of the gastrointestinal tract, which are always present Thus the damage of the muscle fibers herein described may be regarded as nutritional myodegeneration, leaving the interpretation of the term "nutritional" open to discussion

A result difficult to explain is the spotty distribution of most of the changes occurring in my animals, which affected some fiber or a few fibers, while most of the surrounding fibers appeared normal (fig 3) This irregularity may be attributed to the blood supply or to local vascular changes, but from the microscopic examination no conclusions could be drawn which would supply a satisfactory explanation

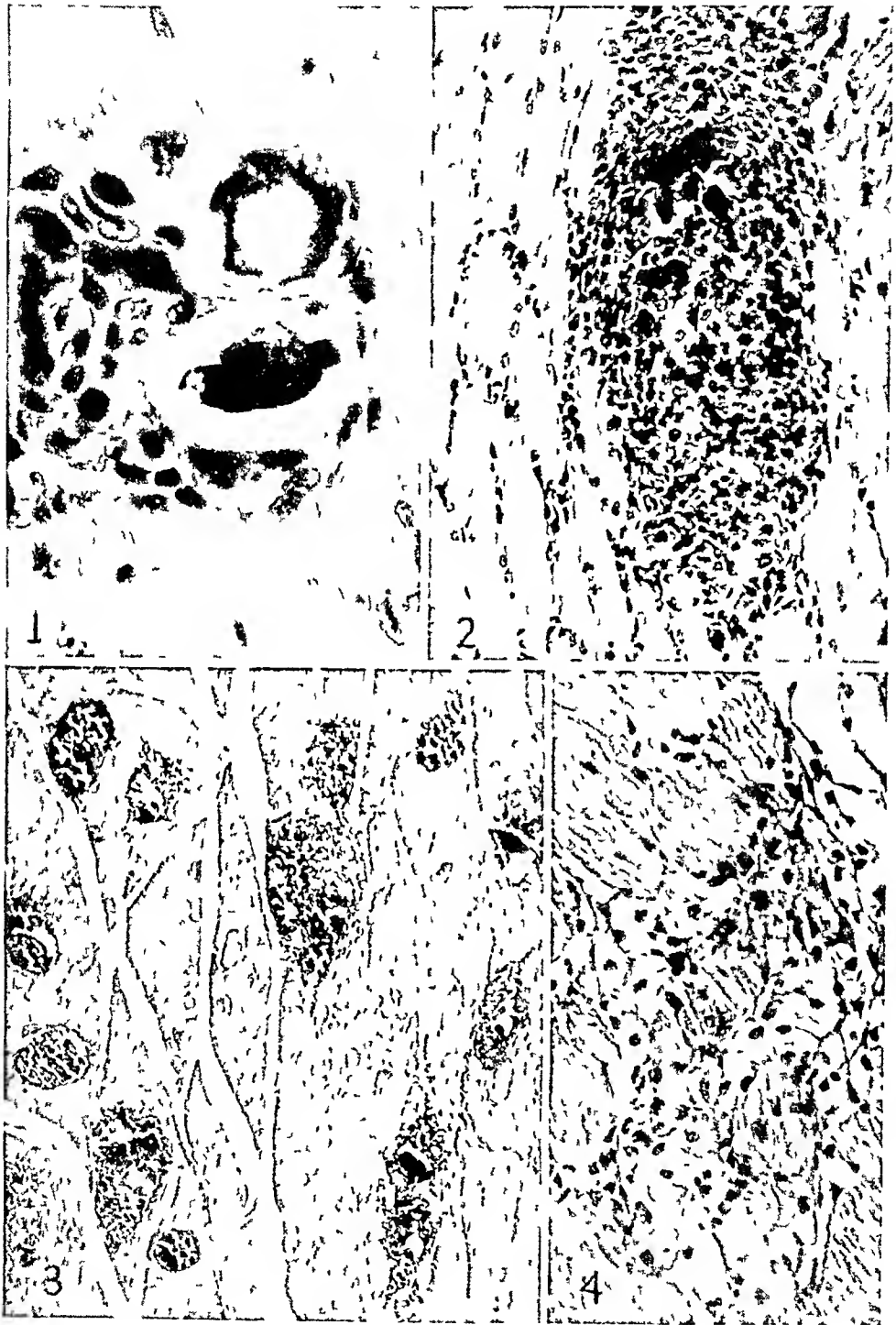
A change which has to be regarded as specific for cholesterol arteriosclerosis is the transformation of muscle corpuscles into foam cells It remains to be shown whether the same pathologic condition may be brought about by other macromolecular substances, such as polyvinyl alcohol Though this change was rather rare, it was distinct In portions of degenerated skeletal muscle fibers the foam cells were crowded against each other and, in some cases at least, filled the sarcolemma completely (fig 4) There may be a simple explanation of the origin

5 Bloom, W, in Maximow, A A, and Bloom, W A Textbook of Histology, Philadelphia, W B Saunders Company, 1942, p 227

6 Goettsch, M, and Pappenheimer, A M J Exper Med **54** 145, 1931

7 Chor, H, and Davenport, R E Arch Path **27** 497, 1939

8 Pappenheimer, A M Am J Path **15** 179, 1939



FIGURES 1, 2, 3 AND 4
(See legends on opposite page)

of the foam cells that they were immigrated histiocytes and were removing a degenerating fiber. But there was no trace of the original muscle nuclei, nor was there any sign of histiocytes that were in the stage of immigration. On the other hand, a gradual transformation of muscle corpuscles into foam cells was seen several times. Finally, foam cells have not been found in skeletal muscle under conditions other than experimental cholesterol arteriosclerosis. When one takes into account the fact that in the course of experimental arteriosclerosis different cell types may turn into foam cells (Hueper², Altschul³), it appears likely that in the cases under consideration the muscle corpuscles which originated by dissociation of the damaged fiber became foamy. If that is the case, it remains to be determined whether these muscle corpuscles actively absorbed the fatty degenerated sarcoplasm or the lipid material from the tissue fluids, or whether they were only fatty degenerated cells and not phagocytes.

The opinion that muscle corpuscles may act as histiocytes has previously been advanced (see review by Mayenburg⁹) but is not generally accepted. According to Pfuhl's experiments with intramuscular injections of trypan blue, muscle corpuscles are not phagocytic. But Forbus¹⁰ reported distinct phagocytic activity on the part of these elements, a fact which I could confirm in hitherto unpublished experiments.

Besides the spotty manner in which the lesions were distributed inside the single muscle, there was some variability depending on the muscle region. The changes were found in muscles of the hindlegs, in the long dorsal muscles, in the diaphragm, in the muscles of the forelegs, in the muscle coat of the esophagus, in the masseters and in

9 Mayenburg, H. V. Die quergestreifte Muskulatur, in Henke, F., and Lubarsch, O. Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1929, vol. 9, pt. 1.

10 Forbus, W. D. Arch. Path. 2: 486, 1926.

EXPLANATION OF PLATE.

Fig. 1—Tongue of a rabbit fed a milk and yolk diet ninety-five days (unretouched photomicrograph, hematoxylin-eosin, $\times 700$). Note detachment and proliferation of muscle corpuscles. The sarcoplasm and the fibrillae are retracted and basophilic.

Fig. 2—Masseter of a rabbit fed a milk and yolk diet one hundred and twenty days (unretouched photomicrograph, hematoxylin-eosin, $\times 200$). A focus of nuclear proliferation is seen with degeneration of sarcoplasm and disappearance of fibrillae. There is little doubt that nearly all the nuclei are proliferated muscle nuclei and not, as may be assumed, nuclei of histiocytes.

Fig. 3—Muscle of a foreleg of the animal whose masseter is shown in figure 2 (unretouched photomicrograph, hematoxylin-eosin, $\times 50$). Note degeneration of the sarcoplasm, with basophilia, and pronounced nuclear proliferation.

Fig. 4—Muscle of a foreleg of the animal whose masseter is shown in figure 2 (unretouched photomicrograph, hematoxylin-eosin, $\times 300$). Foam cells are seen in muscle fibers.

the tongue. The strange fact was that, generally speaking, these lesions appeared earlier and were more pronounced in the muscles of the hindlegs and occurred later and were less accentuated or did not occur at all in the tongue and in the masseter muscles. The occurrence and the intensity of the changes in the other regions ranged between these two extremes. Goettsch and Pappenheimer⁶ in their experiments on nutritional myodegeneration found the tongue and the masseter muscles completely normal. In other experiments Pappenheimer⁸ found only the skeletal muscle of the tongue to be free of alterations.

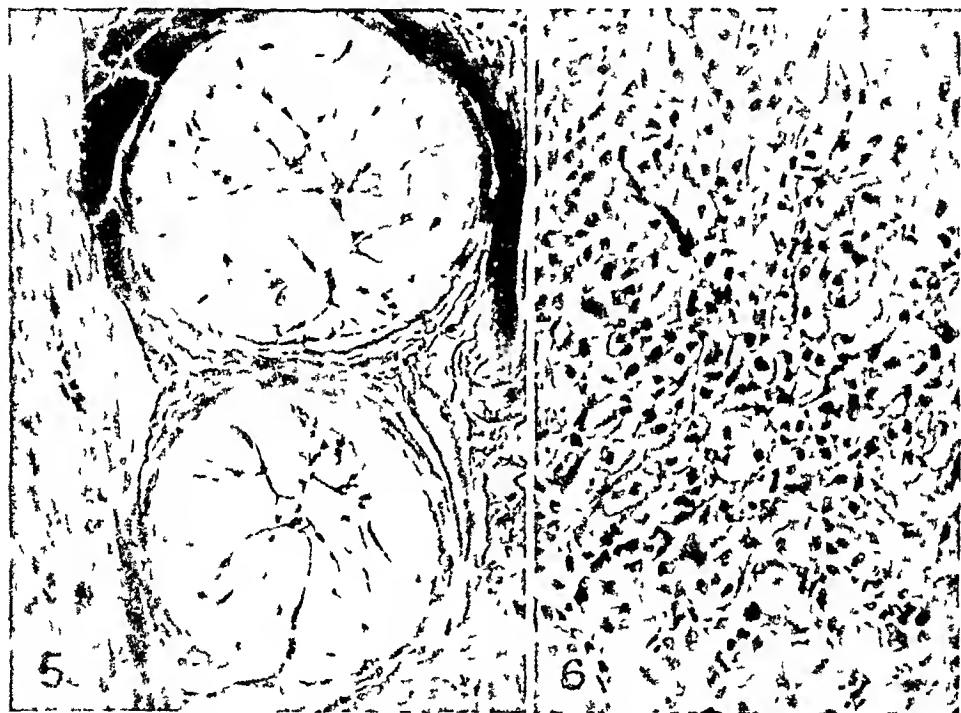


Fig 5—Tongue of a rabbit fed a milk and yolk diet one hundred and twenty-four days (unretouched photomicrograph, hematoxylin-eosin, $\times 175$). Two vessels with "lipoid cushions" in the subendothelial layer are seen. Note that the lumens are only virtual.

Fig 6—Tongue of the same animal (unretouched photomicrograph, hematoxylin-eosin, $\times 250$). Thinned muscle fibers are seen at the bottom and numerous foam cells in the center and at the top (foamy muscle corpuscles?).

In contrast, vascular changes were found much more frequently in the tongue and the masseter muscles and more rarely and to a less extent in the other muscle regions.

The vascular changes were seen mainly as deposits of lipoid material in the subendothelial spaces of the arterioles (fig 5). The deposited material appeared as a light eosinophilic amorphous substance in the hematoxylin-eosin stain, while in Mallory's connective tissue stain the same substance was bluish. Its accumulation in the subendothelial space caused, in many instances, not only narrowing of the lumen but an

increase of the diameter of the vessel and thinning of the media. In contrast to the vascular changes in some other organs or tissues, the cellular reaction to the lipid deposit was slight, only a few foam cells or spindle cells were found in the amorphous masses. The former may have been detached endothelial cells or immigrated histiocytes, the latter, metaplastic endothelial cells or fibroblasts from the media or the adventitia. Quasi-identical vessels were encountered in the heart and in the muscle wall of the stomach.

It is important to note that these vascular changes were not directly connected with the changes in the muscle fibers which have been described. Frequently no vascular changes were evident in muscles with parenchymal lesions, while vascular changes apparently caused no damage to muscle tissue proper. But in a few instances, though distinctly only in the tongue, areas were found in which the marginal zone showed a reduction in width of muscle fibers with no other parenchymal damage (fig 6). In the center was an increase of cells, the majority being of the foam cell type. Whether they were immigrated or autochthonous histiocytes, fibroblasts of the endomysium or foamy transformed muscle corpuscles is open to discussion. The microscopic picture favors the last interpretation, although it does not exclude the possible participation of other cell types. As for the pathogenesis of these foci, I believe that they are caused by insufficiency of the blood supply, due probably to slow occlusion of a blood vessel.

It has already been mentioned that except in the choioid plexus no changes had previously been observed in the vessels of the central and the peripheral nervous system but that I³ was able to provoke them in several animals. This, I suggested, might be due to the fact that in my experiments the yolk had been heated in baking the cake and its cholesterol thus rendered more toxic. This hypothesis, based on the difference in findings between my experiments and those of previous workers in which no heated cholesterol was used, is supported by the observation that gastric tumors may be produced by feeding heated cholesterol (Beck, Kirby and Peacock¹¹). The lesions of skeletal muscle may have been brought about by this same increase of toxicity.

SUMMARY

By feeding rabbits, guinea pigs and golden hamsters a diet rich in cholesterol, changes were provoked in skeletal muscle. They may be grouped into changes of the muscle fibers proper and changes of the blood vessels. In the first group there are varying degrees of intensity (a) waxy degeneration, (b) granular necrosis with separation of the

¹¹ Beck, S., Kirby, A. H. M., and Peacock, P. R. *Cancer Research* 5: 135, 1945.

proliferated nuclei and (c) thinning (atrophy) of the muscle fiber with basophilia and nuclear proliferation. These changes are not considered as specific for experimental cholesterol arteriosclerosis but rather as nutritional myodegeneration.

In several instances foam cells were found inside the sarcolemma, and it is suggested that they are foamy transformed muscle corpuscles.

The vascular lesion consists of a lipoid deposit in the subendothelium of the arteriole with a slight cellular reaction. The vascular lesions are not directly related to the intensity of the damage of the muscle fibers.

Heating of cholesterol may have some bearing on the intensity and the distribution of the lesions in experimental cholesterol arteriosclerosis.

ATHEROSCLEROTIC VALVULAR DISEASE OF THE HEART

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SINCE 1904, when Monckeberg¹ described calcific sclerosis of the aortic valve and attributed it to a degenerative process, numerous opinions have been expressed as to the causation of this lesion, some opposing and some supporting the original contention. Those opposing the view have usually stated the cause to be rheumatic fever.² Sohval and Gross³ have reviewed the problem and have presented good evidence against rheumatic fever in some cases, subscribing to the atherosclerotic nature of the valvular disturbance.

The popular controversy as to the rheumatic or nonrheumatic nature of nodular calcific aortic valvular disease has diverted the attention of pathologists and clinicians from the manifest atherosclerotic changes of heart valves, although these changes are observed with remarkable frequency at autopsy. Most observers have directed their attention to the aortic valves,⁴ although calcification of the mitral leaflets⁵ and of the annulus fibrosus of the mitral valve⁶ have been described occasionally. Hellwig⁷ has recently given a rather careful description of the less severe form of atherosclerosis of the mitral valve, and Rytand and Lipsitch⁸ have reported cases in which complete heart block and cardiac murmurs were associated with calcification of the mitral annulus fibrosus. Radiologists⁹ have often demonstrated their ability to detect calcification in the cardiac valve areas, although their tendency has been to attribute most of it to old rheumatic valvular disease.

From the departments of pathology of the Southwestern Medical College and Parkland Hospital

1 Monckeberg, J G. *Virchows Arch f path Anat* **176** 472, 1904

2 Christian, H A. *J A M A* **97** 158, 1931. Karsner, H T. *Proc Inst. Med Chicago* **15** 62, 1944

3 Sohval, A R, and Gross, L. *Arch Path* **22** 477, 1936

4 Saltykow, S. *Beitr z path Anat u z allg Path* **60** 321, 1915. Margolis, H M, Zielsen, F O, and Barnes, A R. *Am Heart J* **6** 349, 1930

5 Sato, S. *Virchows Arch f path Anat* **211** 238, 1911

6 Giese, W. *Beitr z path Anat u z allg Path* **89** 16, 1932

7 Hellwig, C A. *Am Heart J* **24** 41, 1942

8 Rytand, D A, and Lipsitch, L S. *Arch Int Med*, to be published

9 Sosman, M C. *Am J Roentgenol* **50** 461, 1943

It appears thus that the position of atherosclerosis in the production of changes in the cardiac valves is not fully appreciated. For this reason, a study of autopsy material and clinical records dealing with this problem has been undertaken.

MATERIAL AND METHODS

The records of 500 autopsies were reviewed to determine the incidence of valvular lesions which were due to atherosclerosis. One hundred and one cases in which this type of lesion was demonstrable were found, and it is on these cases that this report is based. The heart was available for further gross study and special microscopic investigation in 63 of these cases.

Particular emphasis was placed on the study of the gross characteristics of the valvular lesions, the distribution of the lesions and the microscopic changes of early and late stages. Data derived from clinical and autopsy studies were arranged for the purpose of determining the relation between the valvular changes and cardiac failure, murmurs, blood pressure, age, sex, race, and atherosclerosis occurring elsewhere in the body.

Blocks for microscopic study were taken from the aortic and mitral valve areas and stained with hematoxylin and eosin and with sudan IV. The following blocks were taken¹⁰—one through the base of the aorta, the posterior aortic cusp, the intervalvular septum and the anterior mitral leaflet, one through the base of the aorta, the left aortic cusp and the left ventricle, one through the posterior mitral leaflet, the left auricular wall and the left ventricular wall, one transversely through the base of the aorta showing the commissure between the right and the posterior cusp. Other sections were taken as indicated by the nature of the gross lesions encountered.

OBSERVATIONS

A high incidence of atherosclerotic changes involving the heart valves was encountered. The actual incidence cannot be determined in the 500 cases reviewed since slight atherosclerosis of the aortic and mitral valves was not always recorded in the protocols. However, in a group of 100 hearts which were reviewed grossly, the incidence of valvular atherosclerosis, including the earliest forms as well as the more advanced lesions, was 63 per cent. Of the total of 500 hearts, 36 (7.2 per cent) were found to have moderately advanced or marked atherosclerotic changes in the cardiac valves.

Gross Appearance of Lesions—Usually the aortic and the mitral valve were both involved. In the earliest stages, however, the mitral valve was altered by slight atherosclerosis while the aortic cusps remained normal. The tricuspid and pulmonic valves were found to have escaped completely. The earliest lesion in the aortic valve was best seen from above. The aortic aspect of all cusps, usually approximately equally involved, was occupied by an opaque yellowish deposit extending onto the valve surface for one third to one half the distance of the valve

¹⁰ Gross, L., Antopol, W., and Sacks, B. Arch. Path. 10: 840, 1930.

and proximally across the sinus pocket to the region of the annulus of the aortic valve. Although some degree of atherosclerosis was almost always present in the first segment of the aorta, it was not usually continuous with that located in the valve itself. This yellow deposit often occurred without any grossly recognizable thickening or rigidity of the cusps, although such alterations were noted in some of the slightly more advanced lesions. The aortic surface only was involved in the process and usually, at this stage, the lipid deposit could not be identified when viewed from the ventricular surface.

Commissural alterations were slight in almost all cases of atherosclerosis of the aortic valve. In about one half of the cases the commissures revealed no change whatsoever. In those instances in which calcium deposition and thickening of the cusps had taken place, the alterations were minor. The most common change consisted of slight agglutination of the lateral edges of two adjacent cusps. This fusion took place for a distance of about 1 mm and was associated with slight yellow thickening of this portion of the cusps. In several cases the base of the aorta showed nodular plaques of atherosclerosis extending into the commissure, thickening and elevating it. Rarely, calcification was observed in these nodular plaques or in the commissure itself. In 8 of the 63 cases of atherosclerotic valvular disease available for gross study, small bridges of firm fibrous-like tissue were found somewhat below the commissural attachment, extending between two cusps. Such findings have been described by Sohval and Gross³ as commissural bridges and are characteristic of atherosclerotic valvular disease, though they are seen in rheumatic aortic valvulitis. The so-called Lambl's excrescences¹¹ were observed on the aortic valves with frequency when atherosclerotic changes of the valve cusps existed.

In a great many cases the cusps of the aortic valve had undergone further alteration consisting of diffuse thickening or sclerosis. This thickening involved primarily the base of the cusps, but in several cases it extended to the free margin. Some degree of rigidity of the cusps was always present in these cases. Rolling of the free margin of the aortic cusps was not seen in any case of atherosclerotic valvular disease, although tension thickening of the margins was frequently observed. Plaques of calcific deposit occurred in most of these cases. They tended to be flat and were located in the basal portions of cusps, on the arterial surfaces. In more advanced calcific lesions there were roughened nodular masses up to 5 mm in size, which projected into the sinus pocket. In 3 cases in which atherosclerotic thickening and fibrosis occurred there were found at autopsy small granular friable gray thrombi attached to the arterial surface, usually overlying an area of calcific deposit where

11 Gunzel Beitr z path Anat u z allg Path 91 305, 1933

the endothelium had been ulcerated owing to the underlying plaque. These were devoid of any bacterial content. In no case was there actual shortening or contraction of the aortic cusps due to atherosclerotic changes.

In 5 cases the lesion of the aortic valve was extreme, leading to stenosis and also insufficiency. In these advanced lesions the calcific deposits were outstanding but still localized primarily to the aortic surface of the cusps, projecting into the sinus pocket region or onto the aortic surface of the cusp. The cusps were greatly thickened and rigid but not appreciably shortened. Commissural changes were impressive and consisted of extensive fusion of the lateral edges, which was associated with formation of large nodular plaques of calcium in the fused cusps. In these cases the left ventricular chamber was dilated and the wall markedly hypertrophied.

In cases of mild atherosclerotic valvular disease the intervalvular septum usually was the seat of focal rounded flat deposits of lipid material. In cases of more advanced disease it had small to large calcific plaques in the subendocardial layers.

The anterior mitral leaflet was more intensely and more commonly involved than the posterior. Corresponding to the milder atherosclerosis of the aortic valve, in a great number of instances the mitral leaflets revealed multiple lipid deposits on the ventricular surface. When the process was slightly more advanced, the leaflets were thickened, especially in their proximal portions, and rigid to some degree. Calcification occurred occasionally in the anterior mitral leaflet, rarely in the posterior, and not as frequently in either mitral leaflet as in the aortic cusps. In a few cases the leaflets of the mitral valve were rendered rigid and thickened by sclerosis and calcification to such a degree that the mitral orifice appeared to have been slightly stenotic and insufficient.

In 10 hearts the annulus fibrosus of the mitral valve was found to be occupied by a more or less complete ring of calcification. In these the mitral leaflets were also the seat of marked atherosclerosis with thickening and some degree of calcification. The calcific ring could be seen located in the base of attachment of the cusp, projecting somewhat inferiorly into the left ventricular wall and onto the ventricular surface of the posterior mitral leaflet. The calcific ring varied from 2 mm to 6 mm in diameter and was thickest about the midportion of the posterior leaflet, becoming smaller toward the anterior leaflet, and disappearing in the base of the anterior leaflet in some cases. The relationship of this calcification of the annulus of the mitral valve to certain clinical findings will be noted later. The distal portions of the mitral leaflets were comparatively thin in these hearts, and the chordae tendineae were thin, revealing no evidence of previous rheumatic fever. Furthermore, his-

tologic study of the valves and of the myocardium of these hearts excluded rheumatic fever as an etiologic factor

Microscopic Observations—The normal histologic structure of the aortic and mitral valves and the intervalvular septum¹² plays an important part in the development of atherosclerosis in these areas. The atherosclerotic changes regularly involve the surface of the aortic and mitral valves which are opposite to the outflow surfaces, that is, the arterial surfaces of the aortic valve and the ventricular surface of the mitral valve. The fibrosa and the adjacent fibroelastic coats of these surfaces, as well as the annulus fibrosus of the aortic and the mitral valve, are the sites of involvement almost to the complete exclusion of other layers or portions of the valves.

The annulus fibrosus of the normal aortic valve consists of a mass of dense, hyaline-appearing fibrous tissue extending a little above the valve ring and ending superiorly at the termination of the medial coat of the aorta. This annulus continues directly into the aortic cusp with the dense, hyaline fibrosa. Both of these structures, that is, the annulus and the fibrosa of the aortic cusp, are covered by the arterial fibro-elastica. The layers of arterial fibroelastica, fibrosa, spongiosa and ventricular fibroelastica are well demarcated in the aortic valve. Beginning just below the ring spongiosa of the aortic valve is the dense fibrous layer, or fibrosa, of the intervalvular septum, also covered by a layer of fibroelastic tissue. The fibrosa of the septum is continued directly into the anterior leaflet, as the fibrosa of this cusp. Elsewhere in the mitral valve there is a distinct annulus quite similar in histologic structure to the annulus of the aortic valve.

It is apparent from the histologic study that the annulus fibrosus of the aortic and the mitral valve, the fibrosa of the two valves and the fibrosa of the septum decrease in cellularity with increasing age. The annulus sometimes assumes the histologic appearance of fibroelastic cartilage in the later age groups. Apart from the changes characteristic of atherosclerosis no apparent thickening or other abnormality can be identified as a part of the process of aging alone.

The earliest stage of atherosclerosis could be seen in the aortic or the mitral leaflets as an extracellular deposition of lipid material. The deposition was so mild in some cases that on gross examination no evidence of it could be detected. In the area of the aortic valve the deposition of lipid material occurred uniformly in the sinus pocket, involving mainly the fibroelastica and the adjacent portion of the annulus. The deposit extended onto the arterial surface of the cusp for a short distance, involving here the fibrosa and the arterial fibroelastica. The deposition was either confined to the fibroelastica or involved the fibro-

12 Gross, L., and Kugel, M. A. *Am J Path* 7 445, 1931

elastica and the superficial layers of the fibrosa. In the anterior mitral and to a lesser extent in the posterior mitral leaflet, early stages of involvement likewise showed histologic evidence that lipoid material was deposited in the outer portion of the fibrosa, and in the ventricular fibroelastic covering. In paraffin sections stained with hematoxylin and eosin these lipoid deposits were characterized by striking paleness, looseness and a foamy appearance. With fat stains, the loose spaces were found to contain large amounts of lipoid substance. Decreased cellularity of the involved coats was apparent in these cases. The lipoid deposit was often found composed of long needle-like crystalline formations resembling cholesterol crystals, arranged at right angles to the surface of the valve. In many cases there was a linear collection of macrophages, which were pale staining owing to contained cholesterol esters, along the arterial fibroelastica of the aortic valve and along the ventricular fibroelastica of the mitral.

These atheromatous lipoid deposits, in the early stage of the process, had produced slight thickening of the valve wall, but there was no other reaction of inflammation or vascularization. In more advanced lesions the lipoid deposit extended along the entire course of the leaflets and there was greater thickening of the valves. It was noted that even with advanced deposition of lipoid material there was no appreciable fibrous proliferation in any of the valve layers, although the fatty deposit itself had led to appreciable thickening and rigidity. In numerous instances scattered fine granular deposits of calcium salts were found in the aortic annulus, but massive calcification of this structure was never observed as it was with considerable frequency in the annulus of the mitral valve.

To be distinguished from these atheromatous lipoid deposits, there were in several cases formations of adipose tissue within the valves. In paraffin sections these were easily recognized by the presence of distinct fat cells with typical large signet ring arrangement. A similar appearance was noted also in the sudan IV stains, and in these the fat deposits took a deeper red stain than did the atheromatous deposits. Characteristic of this type of lipoid deposit of aortic and mitral leaflets was its occurrence without exception in the spongiosa. On some occasions this adipose tissue could be traced in the spongiosa through the valve ring into the adjacent pericardial wedge of fat. Adipose tissue formed in the heart valves, as described, was found with equal frequency in normal hearts and in those with valvular atherosclerosis, and is not considered, therefore, to be an abnormal finding.

A striking feature of severe forms of valvular atherosclerosis was deposition of large calcareous masses. The sites of predilection were in order of frequency first the cusps of the aortic valve, distal to the sinus pocket, about the midportion or in the proximal half of the valve, second, the aortic sinus, overlying the annulus, third, the annulus fibro-

sus of the mitral valve, fourth, the annulus fibrosus of the aortic valve, and finally, the intervalvular septum and the proximal portion of the anterior mitral leaflet. These calcific masses thus appeared at the sites of previous lipid deposits. The calcific deposits occurred on the ventricular surfaces of the mitral valve and the septum, and on the arterial surface of the aortic cusps, involving the fibrosa and the covering fibroelastica in these locations. On decalcification a homogeneous pink-staining acellular matrix remained. The other layers of the valve were essentially intact even in the presence of extensive valvular calcification. Around the borders of calcification there could usually be seen a slight zone of fibrosis and often a slight infiltration of lymphoid cells. In a few cases there was moderate vascularization of the fibrous zone surrounding the calcific deposits.

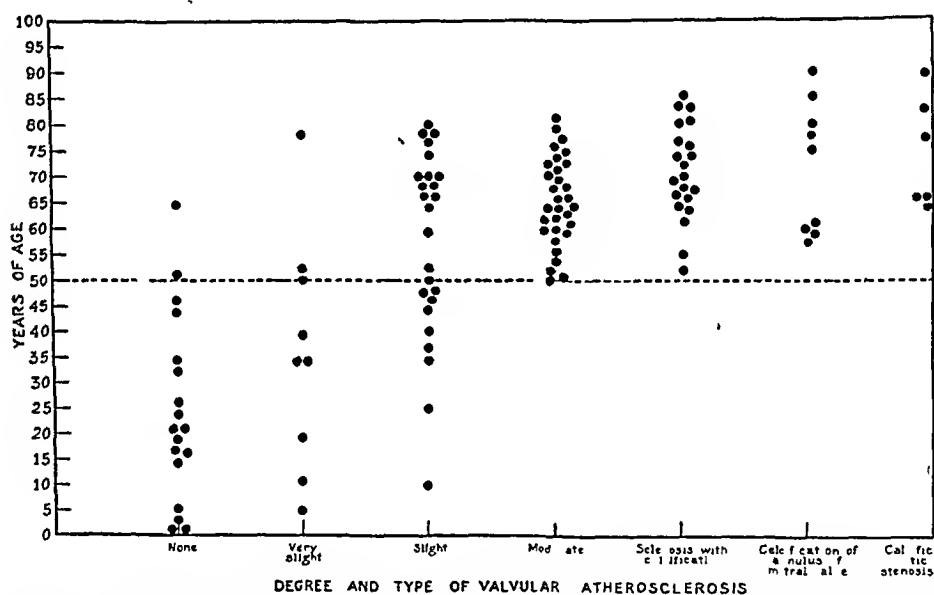


Fig 1—Scattergram showing the relationship of the incidence and the degree of valvular atherosclerosis to age

ACCESSORY CLINICAL AND PATHOLOGIC FINDINGS

Age—It is quite evident that a correlation exists between the age of the patient and the occurrence of atherosclerosis of the aortic and mitral valves (fig 1). In several patients below 10 years of age mild atherosclerosis of the anterior leaflet of the mitral valve was present. In these patients there was usually no accompanying atherosclerosis of the aortic valve or of the aorta. The same was true for most patients with atherosclerosis who were 25 years of age or less. In patients more than 25 years of age mild atherosclerosis of the aortic valve was often present in addition to that of the anterior mitral leaflet. The incidence of atherosclerosis increased markedly after 50 years of age, so that above this age atherosclerosis was almost always present to some degree in the valves.

Among 100 patients in whose case particular care was taken to detect the presence of mild atherosclerosis, there were 2 over 50 years of age in whom no atherosclerosis had occurred in the heart valves. There was no

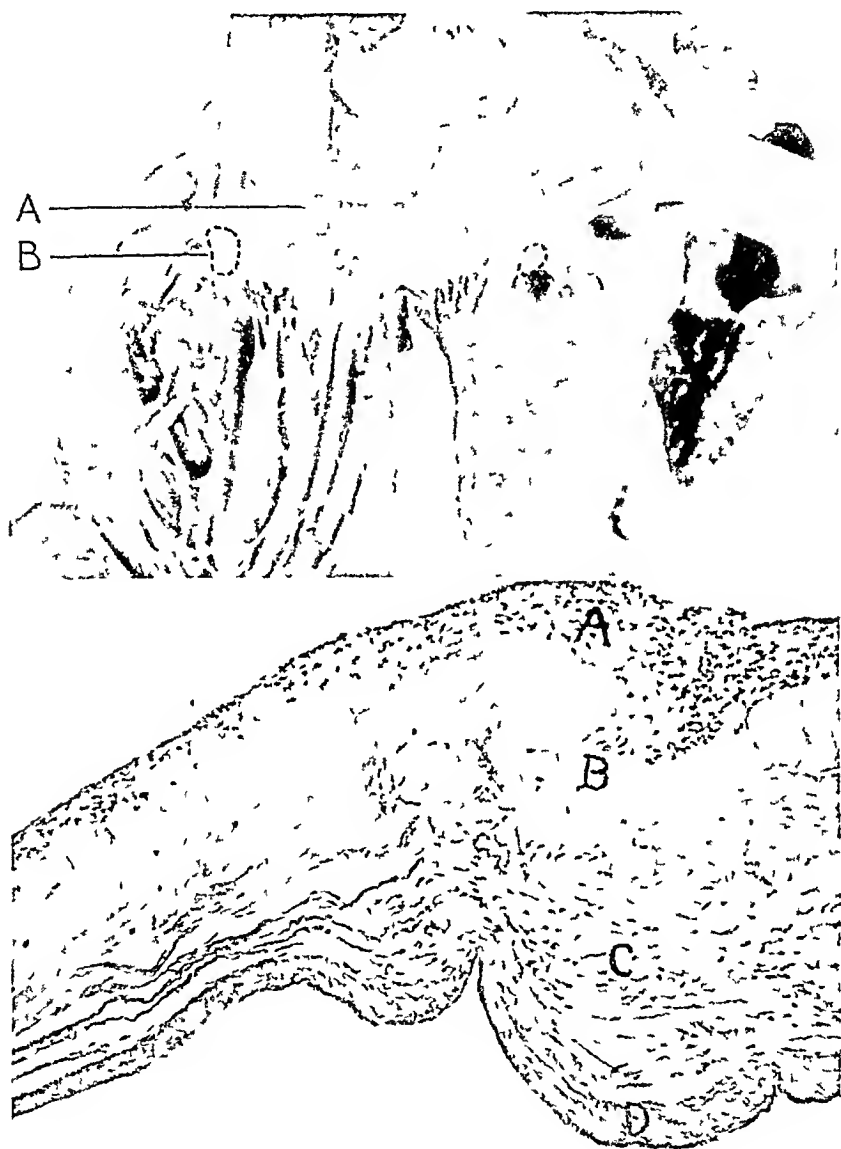


Fig 2—Upper part. Heart with calcification of the annulus fibrosus of the mitral valve. *A* is the contour of the ring as seen through the endocardium at the base of the posterior mitral leaflet. *B* is the cut section of the calcific ring.

Lower part. Aortic cusp with moderate atherosclerosis. Note in the arterial fibroelastica (*A*) the many macrophages containing lipid material and observe the marked atherosclerosis of the superficial portion of the fibrosa (*B*). The spongiosa is entirely normal, as is the ventricular fibroelastica (*D*).

evidence of anything more than slight valvular atherosclerosis in patients under 50. The severity of the atherosclerosis seemed to increase with the age, so that about 42 per cent of patients over 50 were found to have

sclerosis and calcification, calcification of the mitral annulus or aortic stenosis

Race and Sex—The sex incidence in the 500 cases reviewed was 63.2 per cent males and 36.8 per cent females. The racial distribution was 53.2 per cent white persons other than Latin Americans, 40.4 Negroes and 6.4 per cent Latin Americans. Of the patients who had moderate or marked atherosclerotic valvular lesions, 77 per cent were males and 23 per cent females. Eighty and four-tenths per cent were members of the white race other than Latin Americans, 17.6 per cent were Negroes, and 2 per cent were Latin Americans.

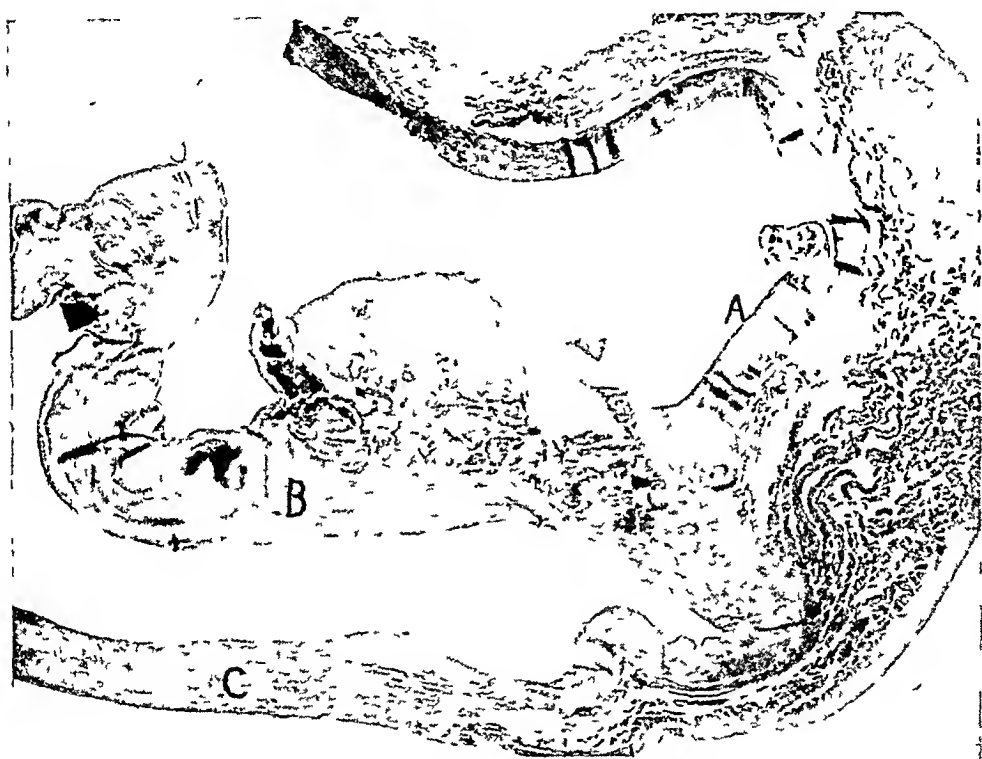


Fig 3—Photomicrograph of the base of the aorta (A), the posterior aortic cusp (B) and the anterior mitral leaflet (C). Marked calcific sclerosis of the aortic cusp and marked atherosclerotic thickening of the anterior mitral leaflet are shown.

Relationship of Hypertension to Atherosclerosis of Cardiac Valves—The criterion for the existence of hypertension in the cases analyzed was systolic pressure above 150 mm and diastolic pressure above 90 mm of mercury or definite arteriolosclerosis of the kidneys. In the majority, both criteria were fulfilled. Of 101 patients with atherosclerosis of the heart valves, 39 had hypertensive disease. In the majority of cases this was classified as essential hypertension. Of 33 patients showing sclerosis with calcification, calcification of the mitral annulus and aortic stenosis, 16 were hypertensive and 17 nonhypertensive, of

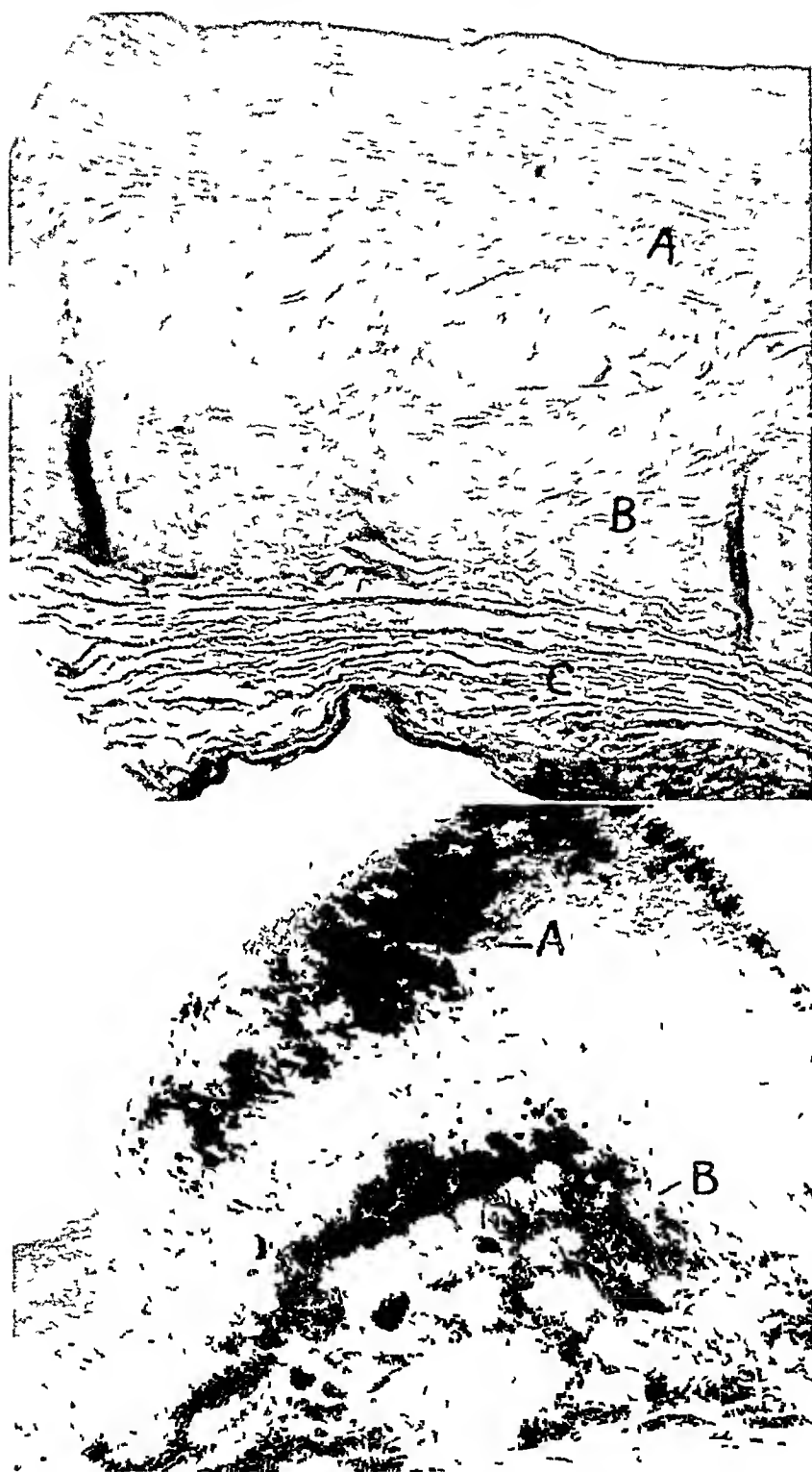


Fig 4—Upper part Moderate atherosclerosis of the anterior mitral leaflet Note the lipoid deposits in the fibroelastica and the superficial layers of the fibrosa (A) The remaining fibrosa shows a decrease in cellularity (B) The spongiosa (C) and the auricular fibroelastica (D) are normal

Lower part Fat stain of an aortic cusp showing lipoid deposits in the fibroelastica and the fibrosa as seen in atherosclerosis (A) Note that the adipose tissue extends into the spongiosa of the aortic cusp (B)

66 who had slight to moderate atherosclerosis, 23 were hypertensive and 43 were nonhypertensive. However, even though these observations might appear to indicate that there is some relation between more advanced valvular atherosclerosis and hypertension, the incidence of earlier ages and hence less hypertension, was considerably greater in those with only slight or moderate valvular atherosclerosis. Correcting for this disparity in age distribution by taking into consideration only patients 50 years of age or over, one found that of those with the more advanced forms of atherosclerosis, 16 were hypertensive and 17 nonhypertensive, as stated, while among the patients with the less severe forms there were 20 who were hypertensive as against 30 nonhypertensive. The difference in the incidence in the two groups is not apparently significant.

Cardiac Murmurs in Cases of Valvular Atherosclerosis—In 21 of the group of 101 cases of valvular atherosclerosis murmurs were described in the clinical records. In the 5 cases in which there was aortic stenosis a systolic murmur was noted at the base of the aorta, while a diastolic murmur was found at the base in 3, a systolic murmur at the apex in 3 and a mitral diastolic murmur was noted in 1 case.

Of the 10 cases in which calcification of the annulus fibrosus of the mitral valve was found, murmurs were recorded in 5. In each of these 5 a systolic apical murmur, usually grade 1 or 2, was noted and in 1 there was a systolic and in 1 a diastolic murmur at the base. In each of the cases in which a systolic apical murmur was described, the mitral leaflets had been rendered rigid and thickened by sclerosis and calcification, in addition to the presence of calcification of the annulus. The ring of calcium was thicker in the cases with murmurs than in those without. In 2 of the cases in which calcification of the annulus fibrosus of the mitral valve was associated with murmurs there was complete heart block. This syndrome has been carefully described and discussed by Rytand and Lipsitch.⁸

Systolic murmurs of low intensity were described in 4 cases in which there was sclerosis with calcification of the mitral and aortic valves (exclusive of the cases in which there were calcification of the mitral annulus fibrosus and aortic stenosis). However, in 2 of these cases anemia of less than 10 Gm of hemoglobin was present, leaving only 2 cases in which the murmurs might be attributed with some certainty to the valvular sclerosis and rigidity.

In the cases of slight or moderate atherosclerosis of the valves, 65 in number, murmurs were heard in 5. The murmur was mitral systolic in each instance. However, in each instance the murmur could be explained on some other basis than the valvular lesion. In 3 cases there was anemia of less than 10 Gm of hemoglobin, in 1 case there was acute

bacterial endocarditis of the mitral valve and in 1 case there was old mitral valvulitis due to rheumatic fever

Relationship of Valvular Atherosclerosis to Atherosclerosis Elsewhere—The fact that the valvular atherosclerosis and the sclerosis occurring in arteries throughout the body were identical in nature suggests that some single underlying factor or group of factors might be at work in the production of these lesions and that a correlation between the atherosclerotic lesions occurring in the various locations could be established. Such is actually the case. As the atherosclerosis of the valves increases in severity, so also does the atherosclerosis of the first portion of the aorta and of the coronary arteries. It should be noted, however, that there is not necessarily, in fact not usually, any direct continuity between the aortic arteriosclerosis and the atherosclerosis of the aortic valve. In fact, in several instances aortic arteriosclerosis was absent or slight, while the valvular atherosclerosis was marked.

COMMENT

Valvular atherosclerosis is a common lesion, although it does not often lead to the production of clinical manifestations. In 7 of 500 cases with autopsy, however, death was actually due to some form of atherosclerotic valvular disease. In 5 of the 7 cases calcific aortic stenosis was present, in 2 there was complete heart block due to calcification of the annulus fibrosus of the mitral valve. In several other cases cardiac murmurs without impairment of cardiac function were produced. Although minor embolic phenomena were not observed in any of the cases of this series, they might be expected to accompany atherosclerotic valvular disease, since thrombi were observed on the altered valves in a few cases. I have never observed subacute bacterial endocarditis occurring on an atherosclerotic valve, nor do I know of any recorded instance of it.

The most common valvular atherosclerosis is that of simple deposition of lipoid material. Sclerosis with thickening and calcification of the valves occurred in an appreciable but smaller number of the cases. Calcific aortic stenosis comprised a small group, but one of the utmost clinical importance, while calcification of the mitral annulus fibrosus was present in 10 cases. In 2 cases of the latter group complete heart block was produced. It is proposed that these various types of valvular lesions be referred to as forms of atherosclerotic valvular disease.

As to the genesis of atherosclerosis of the heart valves, the same general factors which enter into the development of arteriosclerosis elsewhere seem to be of importance.¹³ It has been noted that atherosclerosis

13 Leary, T. Arch. Path. 23 185, 1943

of the valves is closely related to age. Hypertension, if it has any effect at all, simply increases the severity of the lesion, and certainly is not the primary condition. The fact that the atherosclerosis is characteristically distributed with great constancy in the annulus fibrosus and in the fibrosa of the mitral and aortic valves would seem to be related in some way to causal or contributing influences. It has been noted that these regions of involvement in the connective tissue, which, even in the young person is relatively acellular, dense and devoid of blood vessels, decreases in cellularity with age. Certainly such an alteration in cellularity and in metabolic activity would be expected to predispose to deposition of calcium and perhaps also of lipid material. In other regions of the body it is not unusual to find lipid material deposited in the substance of dense hyaline fibrous tissues, as in connection with old productive inflammation in some instances, or in the common hyaline plaques which occur in the splenic capsule, or in the dense scarlike tissue which makes up the wall of an old hydrocele sac. One might expect, therefore, a similar deposition of lipid material to take place in the dense hyaline tissue of the annulus fibrosus of the heart valves and in the fibrosa of the cusps. This deposition would further be expected to vary in intensity with the amount of cholesterol or other lipid substances circulating in the blood, as it appears to do in arteriosclerosis.¹⁴

The distribution of the atheromatous deposits described suggests that tension and vibration might be factors concerned in the genesis of atherosclerosis of the valves. The aortic surface of the aortic cusps and the ventricular aspect of the mitral leaflets are the surfaces which are subjected to the greatest pressure and on which the greatest vibrating effect of the moving columns of blood would be exerted.

It appears that sex and race are factors of minor importance in the occurrence of atherosclerosis of the cardiac valves. There is some suggestion in the material presented that there is a slightly greater incidence in males than in females and that the disease occurs with greater frequency in the white race than in the Negro. Although Martens¹⁵ and also Rytand and Lipsitch⁸ reported calcification of the annulus fibrosus of the mitral valve to be more common in women than in men, in the 10 cases reported here, males predominated 6 to 4.

The similarity of the problem of valvular atherosclerosis to that of generalized arteriosclerosis is further suggested by the correlation shown to exist between the occurrence of aortic and coronary arteriosclerosis and that of valvular atherosclerosis.

14 Hirsch, E. F., and Weinhouse, S. *Physiol Rev* **23** 185, 1943.

15 Martens, G. *Beitr z path Anat u z allg Path* **90** 497, 1932.

SUMMARY

Atherosclerotic valvular disease occurs with considerable frequency and occasionally gives rise to significant clinical findings. These consist of aortic stenosis, heart block from calcification of the annulus fibrosus of the mitral valve, and precordial murmurs in some cases of sclerosis with calcification of the aortic and mitral leaflets. Mild and moderate valvular atherosclerosis without associated clinical manifestations is exceedingly common after 50 years of age. Hypertension was present in the cases of more intense valvular atherosclerosis slightly more frequently than in those in which the lesions were mild.

On gross examination atherosclerosis of the mitral and aortic valves was characterized by involvement of the aortic sinus pocket and the arterial surface of the adjacent proximal segment of the valve cusp, the intervalvular septum and the ventricular surface of the proximal portion of the mitral leaflets, particularly the anterior. An opaque yellow deposit was characteristic of earlier stages, followed in some cases by thickening and rigidity of the leaflets, calcification, stenosis and the formation of a calcific ring around the mitral valve. Microscopically, the earliest change was the deposition of lipid material occurring in the fibroelastic coverings of the mitral and aortic leaflets, in the adjacent fibrosa and in the annulus fibrosus of the aortic and mitral valves. Slight calcific deposits were seen in the annulus of the aortic valve as was calcification in the regions of the lipid deposits. Fibrosis and slight chronic inflammation were noted around the areas of calcific deposits. The valvular layers remained fairly well intact even in the presence of extensive calcific deposits. Macrophages containing cholesterol esters were found near the deposits of cholesterol in the heart valves.

Factors considered to be related to the development of atherosclerosis of the heart valves were age, hypertension, the physiologic decrease in cellularity of the annulus and of the fibrosa of the aortic and mitral valves and the effect of tension and vibration on certain portions of the valves.

REACTION OF THE RETICULOENDOTHELIAL CELLS TO SUBCUTANEOUS INJECTIONS OF CHOLESTEROL

Experimental Animals Mice

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A SERIES of studies has been carried out in recent years with the aim of obtaining information concerning the responses of tissues, particularly those of the reticuloendothelial system, to specific lipids by correlation of histochemical technics. The subcutaneous area was chosen as the preliminary site of investigation with each lipid, before proceeding to the more complicated reactions of the system as a whole, because of the relative freedom from rapid admixture with other lipids which may be maintained in that locality and because of the relative simplicity of normal subcutaneous tissue and, therefore, the ease with which infiltrations and reactions of cells can be followed in that region and applied to the interpretation of events in more complex regions.

The studies to date have included the reactions to various classes of lipids which do not contain cholesterol. The reactions to synthetic triglycerides,² to individual phospholipids^{3a} and to galactolipids³ have been presented. Except in the case of the galactolipids, the reactions were found to involve chiefly the cells of the reticuloendothelial system, and the morphologic aspect of these cells varied characteristically with each class of lipids and, at times, even with the individual members of a class. Investigation of the reactions to the groups which contain cholesterol, i. e., cholesterol and its esters, with fatty acids, now seems indicated. The present study concerns the reactions to cholesterol.

MATERIAL AND METHODS

Chemically pure cholesterol (Coleman & Bell) was used for these studies. It was kept as a stock solution in alcohol (0.10 Gm to 100 cc of 95 per cent alcohol) and was injected as a colloidal suspension in a 5 per cent dextrose solution. Fresh suspensions were prepared for each injection by slowly dropping the

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1 Footnote deleted by the editor

2 Gray, M. E. *Am J Anat* **67** 361, 1940

3 Tompkins, E. H. (a) *Bull Johns Hopkins Hosp* **70** 55, 1942, (b) *South M J* **33** 154, 1940

4 Footnote deleted by the editor

desired aliquot of stock solution into the desired amount of dextrose solution over a boiling water bath. The dextrose solution was stirred constantly as the solution of cholesterol was added, and agitation was continued until all odor of alcohol had disappeared. Boiling distilled water was then added to bring the volume of dextrose solution back to the original, and the emulsion was drawn into a tuberculin syringe and allowed to cool. For experiments to be terminated within three days after the injections the concentration of cholesterol in colloidal suspension was 0.05 per cent, for experiments to be terminated between three and four days, 0.10 per cent, for experiments to be terminated between five and twelve days, 0.15 per cent, and for experiments to be terminated later than fourteen days after the injections, 0.20 to 0.25 per cent. The 0.05 and 0.10 per cent emulsions were clear, the more concentrated ones, slightly opalescent. Suspensions with cholesterol in 0.05 and 0.10 per cent concentrations could be maintained in the syringe indefinitely. Under the microscope hanging drop preparations of the suspensions presented separate pinpoint specks, like chylomicrons, in brownian movement and without aggregates. These lent a dull glow to the drop in polarized light but did not appear as sharply defined globules or crystals. After an hour or longer the specks became converted into small aggregations of extremely fine needle-like crystals, which were sometimes definitively anisotropic.

The injections were made subcutaneously in the lower abdominal and lumbar areas of mice of the Wistar strain that had long been interbred in the laboratory. Adults of both sexes were used (the females nonpregnant). One-half cubic centimeter of the suspension was given in each injection. No evidence of irritation was observed. The two areas of any given mouse were injected at different intervals, which were so spaced that the tissue reactions of the same mouse would be representative of alternate periods of examination. Examinations were made at hourly intervals up to twelve hours after the injection, then every two hours up to twenty-four hours, every twelve hours up to seventy-two hours, every twenty-four hours up to twelve days and finally, every forty-eight hours up to twenty-one days after the injection.

The mice were kept under the routine laboratory conditions (Purina Chow,⁵ oats, lettuce and water ad libitum) until such time as examination was desired. At that time the experiment was conducted essentially in the manner described by Gray² after injections of triglycerides. An anesthetic dose (5 mg.) of pentobarbital sodium was given intraperitoneally in 1 cc. of saline solution. When anesthetized, the mouse was exsanguinated by sterile cardiac puncture. The blood serum was obtained for use in succeeding experiments. The areas of injection were incised, and the skin was pushed back by blunt dissection. Tiny snips of connective tissue from the underlying areas were filmed on cover slips by

5 The Ralston Purina Company states that the ingredients of the chow are meat meal, dried skim milk, riboflavin, carotene, cod liver oil, brewers' yeast, wheat germ, corn grit, wheat cereal, corn cereal, dried beet pulp, molasses steamed bone meal and iodized salt. The chemical analysis shows

| | Crude, % | Digestible, % |
|-----------------------|-------------|---------------|
| Protein | 23.0 | 19.0 |
| Fat | 5.0 | 4.7 |
| Fiber | 4.0 | |
| Ash | 7.0 | |
| Nitrogen free extract | 54.0 | 48.0 |
| Moisture | 7.0 | |
| | <hr/> 100.0 | <hr/> 71.7 |

the aid of teasers. A few of these were immediately moistened with serum from a different mouse, superimposed on slides, rimmed with petrolatum and left in the incubator at 37 C until stained supravitaly. The serum was stained with neutral red just before use by adding 0.5 cc to a tube in which 0.04 cc of a saturated solution of neutral red in absolute alcohol had been previously evaporated to dryness. The other films were air dried and boxed for staining without further fixation.

The supravitaly prepared films were counted differentially at 37 C and were later examined under polarized light at room temperature. The following features were recorded at the time of these counts: the presence, the site and the character of crystals of cholesterol, the presence, the relative refractivity, the size and the cytoplasmic location of droplets of lipid, the presence of intracellular debris, the color, the refractivity, the size, the shape and the cytoplasmic location of the droplets stained with neutral red.

The fixed films were exposed to 1 per cent Nile blue sulfate in water, to Sudan IV (in 70 per cent alcohol at 37 C according to the technic described in Lee's "The Microtometist's Vade Mecum"^{6a}), to osmic acid gas by suspension of wetted films over a 1 per cent solution of osmic acid in water, to the Schultz technic for cholesterol as described by Lee^{6b} and to digitonin with examination by polarized light for differentiation of cholesterol from its esters, according to the technic of Leulier and Revol.⁷ The examination of these films was made first from mounts in glycerin jelly. The jelly was then removed with water, and control observations were made after mounting in balsam in order to determine, by virtue of the xylene present, whether failure to stain with a specific stain indicated merely absence of lipid or the character of the lipid present. The films treated with digitonin were later treated with ether in order to differentiate between the anisotropism due to the digitonin complex with cholesterol and that due to the presence of cholesterol esters.

The films stained with Sudan IV were counterstained with Ehrlich's hematoxylin, and those exposed to osmic gas, with carmine. These films plus those stained with Nile blue sulfate afforded satisfactory observation of nuclear characteristics for correlation with the various cytoplasmic observations.

A Bausch and Lomb polarizing attachment was used for all studies with polarized light. This was used in conjunction with a monocular microscope equipped with a fluorite oil immersion lens.

As the reaction to the injections progressed, it was found that the most comprehensive understanding of the sequence of events was to be obtained at the periphery of masses of cells rather than within such masses. The early reactions were diffuse and generalized, and scattered foci of crowded cells began to develop only after the reactions had been under way for some time. These foci comprised mixtures of primary and secondary reactions, the secondary reactions included degeneration of cells in the packed centers, appearance of autolytic products of degeneration, new reactions to depositions from degenerate cells, continuous filling of cells with cholesterol esters due to continuous exposure to organized crystals of cholesterol and increase of webs of fibrous tissue. The foci, therefore, did not present an uncomplicated sequence of events in the reactions to colloidal suspensions of cholesterol alone. The peripheries, on the other hand, presented a changing array of reactions that seemed to follow one another in orderly, uniform

⁶ Lee, A. B. *The Microtometist's Vade-Mecum*, ed. 10, Philadelphia, P. Blakiston's Son & Co., 1937, (a) p. 283 and (b) p. 284.

⁷ Leulier, A., and Revol, L. *Bull. d'histol. appliq. a la physiol.* 7: 241, 1930.

sequence Once foci developed, therefore, most of the observations and all of the differential counts were made along several axes from a focus

Controls consisted of films from equivalent areas of untreated mice and from mice that had been given at various intervals injections of the menstruum used for the experimental injections, i e, the 5 per cent dextrose solution The control films were stained, examined and counted in the different ways employed for the experimental films

EXPERIMENTAL DATA

The terms "tissue clasmatoocytes" and "macrophages" are employed in the sense in which they were used by Gray² Briefly, the term "tissue clasmatoocytes" refers to the large phagocytic mononuclear cells which are normally present in connective tissues, and the term "macrophages" refers to the phagocytic mononuclear cells which develop from smaller cells that enter from the capillaries under sufficient stimulus Other than these two, it is believed that all other cytologic terms to be employed are uniformly understood, although the cells themselves cannot be uniformly differentiated by different technics

While differential counts were made from both the living and the fixed films of all experiments, these data have been omitted in favor of summarized statements concerning only key points in the experiments The counts served, however, to indicate the times at which characteristic modifications occurred most frequently in the areas of reaction, and the necessity for accurate differentiation in the course of the counts required careful analysis of the morphologic character of every cell in the field

Normal connective tissues in the areas under study contain few types of cells, and they are almost uniform from the point of view of morphology Mast cells, fibroblasts and tissue clasmatoocytes are the constant inhabitants, neutrophils and lymphocytes occur infrequently and clumps of eosinophils at times The tissue is like that found by Gray² in the guinea pig except that mast cells occur more frequently and the ratio of fibroblasts to clasmatoocytes is higher (average 4:0)

The clasmatoocytes of normal tissue are usually elongated and present in scattered groups They contain various-shaped, medium-sized deposits of neutral red with a neutral reaction The fibroblasts contain scattered, extremely fine drops of neutral red Neither cell contains refractive drops of fat in living preparations and neither is anisotropic The clasmatoocytes are finely stippled with red after exposure to sudan IV but contain no larger deposits and do not react to the other stains for lipids The fibroblasts do not react to any of the stains for lipids

An attempt has been made to simplify the description of the reactions of the tissue clasmatoocytes and macrophages observed after the injections of suspensions of cholesterol by presenting a schematic diagram in figure 3 The periods of time stated in the diagram are didactic and represent merely the intervals after injection at which the given morphologic changes occurred most frequently It must be remem-

bered, however, that any given morphologic change occurred and regressed gradually and that cells in other stages of metamorphosis were always present. The diagram indicates merely the period at which cells with a given morphologic character occurred in greatest proportion.

All statements of time have been made to refer uniformly to the interval extending from "the time of injection." Repetition of this phrase is therefore omitted in those places where it would otherwise be desirable for the purpose of scientific certainty.

The areas of injection were edematous for about three hours, and both fibroblasts and tissue clasmatocytes contained large light-colored "fluid vacuoles" in addition to the usual denser deposits of neutral red. The latter were somewhat less sharp than normal. The edematous background glowed diffusely under polarized light, but otherwise there was no evidence of the injected cholesterol. Cholesterol was at times found to be aggregated on clumps of fibrin in films stained with digitonin, but usually it was so scattered and in such minute droplets as to fail detection.

The edema had subsided by the end of three hours, and infinitely fine acicular crystals were found scattered separately throughout the field. Great numbers of these adhered electively to the surface of the tissue clasmatocytes, but at no time did they adhere to mast cells or fibroblasts. The field remained diffusely anisotropic under polarized light up to twelve hours. The crystals were apparently too delicate to be seen as discrete anisotropic particles in that light. By twelve hours they began to show as discrete anisotropic rods (fig 1, *A* and *B*).

Neutrophils began to enter from the vessels by the end of three hours, and the tissue rapidly became filled with them. They wove in and out between the free acicular crystals of cholesterol, but at no time did they acquire crystals. They began to degenerate and to acquire drops of sudanophilic fat at nine hours. They were never anisotropic and did not react to the other lipid stains. Most of them were degenerating at eighteen hours, and all had disappeared within sixty hours. They were somewhat less plentiful in the areas injected with dextrose solution, but otherwise the reaction was similar. Their presence in the experimental areas was obviously not directly concerned with the absorption and removal of the cholesterol except so far as the fat liberated by their death may have been of significance as a source of fatty acids.

During this period the fibroblasts exhibited many medium-sized deposits of neutral red and at times "fluid vacuoles" (fig 1, *B*). In the supravital preparations they also contained many tiny isotropic drops of fat (fig 1, *C*), which at times stained with sudan. They reacted similarly, but to lesser degree, after injections of dextrose solution. They were never found to contain cholesterol. Their content of neutral red was relatively normal again at sixty hours, but they continued to

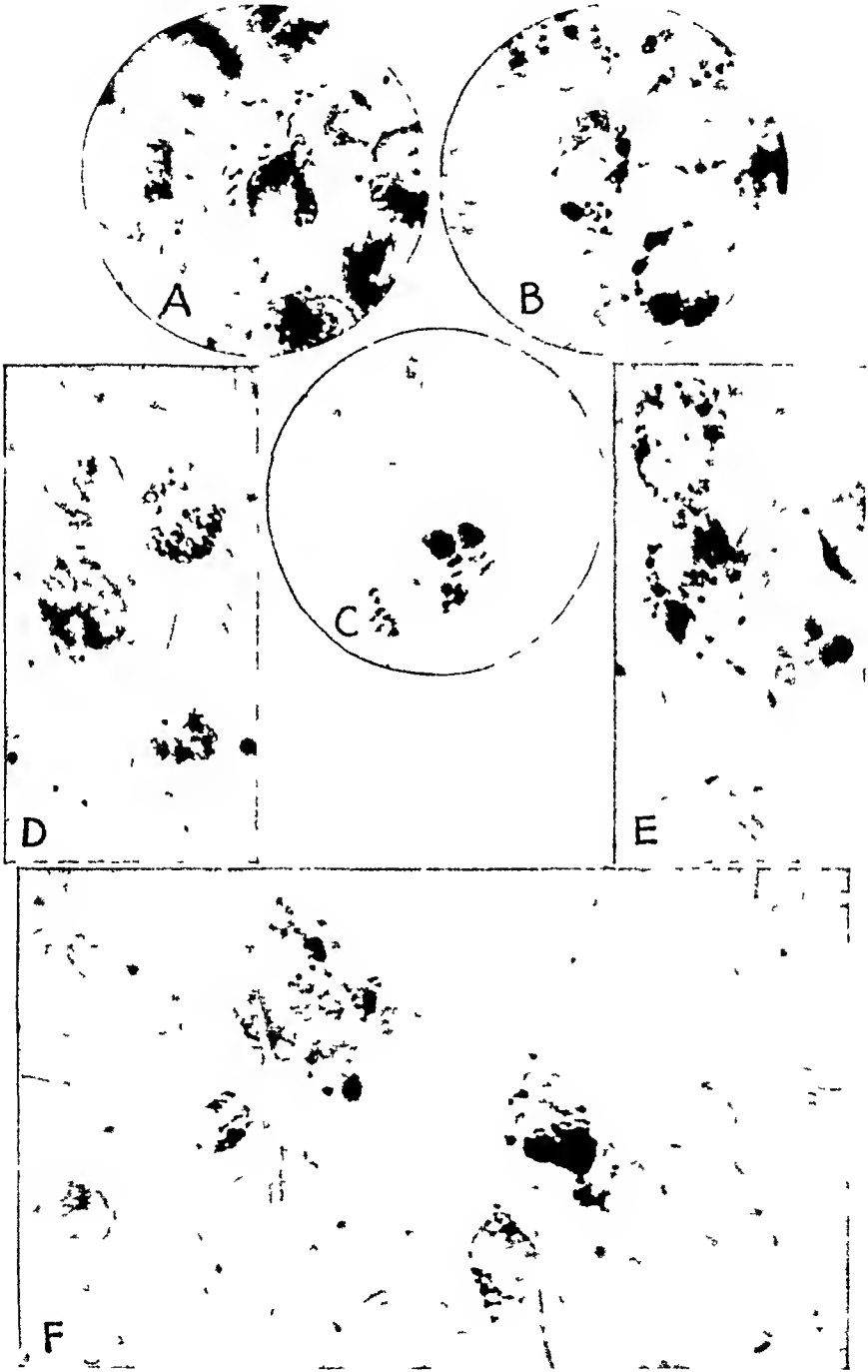


Figure 1
(See legend on opposite page)

have fine droplets of fat for eight to ten days. They often multiplied into dense capsules about foci of packed cells from that time on, since the initial reactions had subsided, this response seems secondary to autolysis and deposition of crystalline material within the foci, and not a part of the primary reaction to a colloidal suspension of cholesterol *per se*.

Mast cells took no part in the reaction. Eosinophils appeared in great numbers in some of the experiments but were so lacking in others that correlation to the injections of cholesterol seems unjustified, despite the frequent association of cholesterol and eosinophils in clinical material.

Up to twelve hours, then, the only cell that was specifically concerned with the cholesterol was the tissue clasmatocyte, and it was literally peppered with discrete acicular crystals. Neutrophils entered the area at three hours, but at no time concerned themselves with the cholesterol. Monocytes began to enter the area at nine hours. They were small and very motile and contained medium amounts of neutral red (fig 1, *F*). They, too, wandered among the crystals of cholesterol without attracting them. They exhibited rapid increase in their own

EXPLANATION OF FIGURE 1

Photomicrographs of supravitaly stained films of connective tissue from mice into which colloidal suspensions of cholesterol had been injected subcutaneously at various periods preceding the examinations. Deposits stained with neutral red appear gray or black, the depth being dependent on the intensity with which they stained. Drops of fat appear as rimmed white circles. Crystals appear as tiny dark or light specks or quadrangles, their color and shape being dependent on the focus at which they were viewed. $\times 781$

A Mouse 94 Abdomen. Cholesterol in colloidal suspension was injected eighteen hours previously. Acicular crystals of cholesterol can be seen scattered throughout the area. Many adhere to the surfaces of the macrophages which had entered the field. The macrophages were small and easily distinguished from rounded tissue clasmatocytes at this stage.

B Same as *A*. Two tissue clasmatocytes may be seen. Acicular crystals of cholesterol are adherent to the upper one. Both had acquired large, dark deposits of neutral red and a few vacuoles of isotropic fat. Fibroblasts are shown in the background. They contained considerable amounts of neutral red and droplets of isotropic fat but were devoid of crystals of cholesterol.

C Mouse 94 Back. A colloidal suspension of cholesterol was injected twenty-four hours previously. A tissue clasmatocyte and a fibroblast are shown. The deposits of neutral red in the former were large and varied in color. Droplets of anisotropic fat were scattered at random in the cytoplasm. The fibroblast, at the top, contained isotropic fat only.

D Same as *C*. Monocytes are seen developing into macrophages. They have scattered acicular crystals on the surfaces and a few drops of isotropic fat in the cytoplasm.

E Two tissue clasmatocytes, which are in the same field as *D*, are shown. They contained considerable neutral red and vacuoles of anisotropic fat and were rounding. A few crystals adhered to the surfaces.

F Same as *C*. A cluster of monocytes which are younger than those shown in *D*, crystals rarely adhered to the surfaces of such monocytes. The macrophage and the tissue clasmatocyte in the same field are similar to those illustrated in *A* and *B*, respectively.

size and in the number and the size of the deposits of neutral red (fig 1, *D*) By eleven hours many contained scattered large drops of isotropic fat and by twenty hours had developed into macrophages (fig 1, *A* and *F*) From then on they began to be as heavily coated with discrete fine acicular crystals of cholesterol as were the tissue clasmatoocytes

In the meantime, from about three hours after the injection the size and the number of neutral red deposits in the clasmatoocytes increased rapidly until deposits of all sizes and shades of red filled the cells (fig 1, *B*, *C*, *E* and *F*, also fig 3, 1° to 22°) They soon became much darker, i e, more acid in reaction, and more refractive The cells often rounded and became apparently free By six hours they also had large refractive drops of isotropic fat scattered at random among the deposits of neutral red, and often cellular debris The crystals adherent to their surfaces in great numbers broadened slightly and often became aligned somewhat in parallel, so that two or three lay side by side By fourteen hours the crystals were definitely broader and thicker and often appeared as well defined anisotropic rods By this stage many of the drops of lipid within the cytoplasm had become anisotropic By twenty-two hours the crystals on the periphery of the clasmatoocytes had so thickened that they had the shape of tiny grains of rice, and all of the isotropic drops in the supravital preparations had become replaced by anisotropic drops and exhibited the cross of polarization indicative of fluid crystals These drops had also acquired a waxy consistency and appeared less refractive than the earlier isotropic drops At this stage they stained faintly with sudan IV and osmic acid

While these changes were occurring in the rounded clasmatoocytes, the macrophages that were continuously developing from monocytes assumed more and more the same characteristics Once they acquired the crystals of cholesterol on their surfaces, they pursued a course similar to that described for the tissue clasmatoocytes, and by thirty-six hours after the time of the injection it became increasingly difficult to differentiate macrophages that had rounded from preexisting clasmatoocytes and macrophages that had arisen by metamorphosis of infiltrated monocytes By that time all crystals had disappeared from both types of macrophages and from the field, both types of macrophages contained debris and large refractive drops of lipid which did not stain supravitaly, which now tended to be peripherally located, which contained beautiful crosses of polarization and which stained fairly deeply with sudan IV and faintly with osmic acid, both types of macrophages were swollen with a tremendous number of vacuoles of neutral red, which were now very dark and refractive and which were beginning to decrease in size, although most of them were still large

From this point on the reaction is obviously related to the events that took place in these cells. Monocytes continued to enter the field for seventy-two hours and to be converted through the stages leading to this type of macrophage, tissue clasmatoocytes which were not already rounded and free eventually became so and merged indistinguishably with their fellow macrophages. The progression of events, however, is represented by the changes which took place in those cells which had already become macrophages and which were filled with deposits of neutral red and with anisotropic drops. The description of the macrophages from now on, therefore, is indiscriminate of their original source.

While free crystals had completely disappeared from both the field and the macrophages thirty-six hours after the injections, the cells continued to acquire increasing numbers of anisotropic drops, which eventually became arranged several layers deep in the periphery of the cytoplasm (fig 2, *G* and *H*). These drops were waxy, brilliantly anisotropic and jeweled with crosses under polarized light, they now stained deeply with sudan, slightly with osmic acid and not at all with Nile blue sulfate, they reacted in the Schultz test and were largely removed by ether after treatment with digitonin. These anisotropic drops occurred in greatest abundance seventy-two hours after the injection, they began to lessen in number during the next two days (fig 3, 36° to 4-5 *d*).

The vacuoles stainable with neutral red, in the meantime, became displaced centrally as the anisotropic drops took up a peripheral position, and at the same time they began to decrease in size and to increase in number and uniformity. This change was marked by forty-eight hours, still more marked by seventy-two hours, the period when the anisotropic drops were most abundant (fig 2, *G* and *H*), and most marked at four and five days, the period when the anisotropic drops were rapidly decreasing (fig 3, 36° to 4-5 *d*). At this stage the deposits of neutral red approached, but never attained, the fineness of the deposits in typical epithelioid cells. By the sixth and seventh days the drops of lipid had decreased impressively and had lost the cross of polarization but were still anisotropic, the deposits of neutral red were as fine as those of the two preceding days but had also decreased greatly in number. From the seventh day the events were regressive in character (fig 2, *I* and fig 3, 5-10 *d*). All fat and the fine, dark deposits of neutral red soon disappeared and were replaced by scattered deposits of neutral red which were more neutral in reaction, larger, angular and more like the deposits in normal tissue clasmatoocytes (fig 2, *J*). The cells elongated and decreased in number. By this time their only reaction to the fat stains consisted of stippling with sudan IV similar to the stippling of tissue clasmatoocytes.

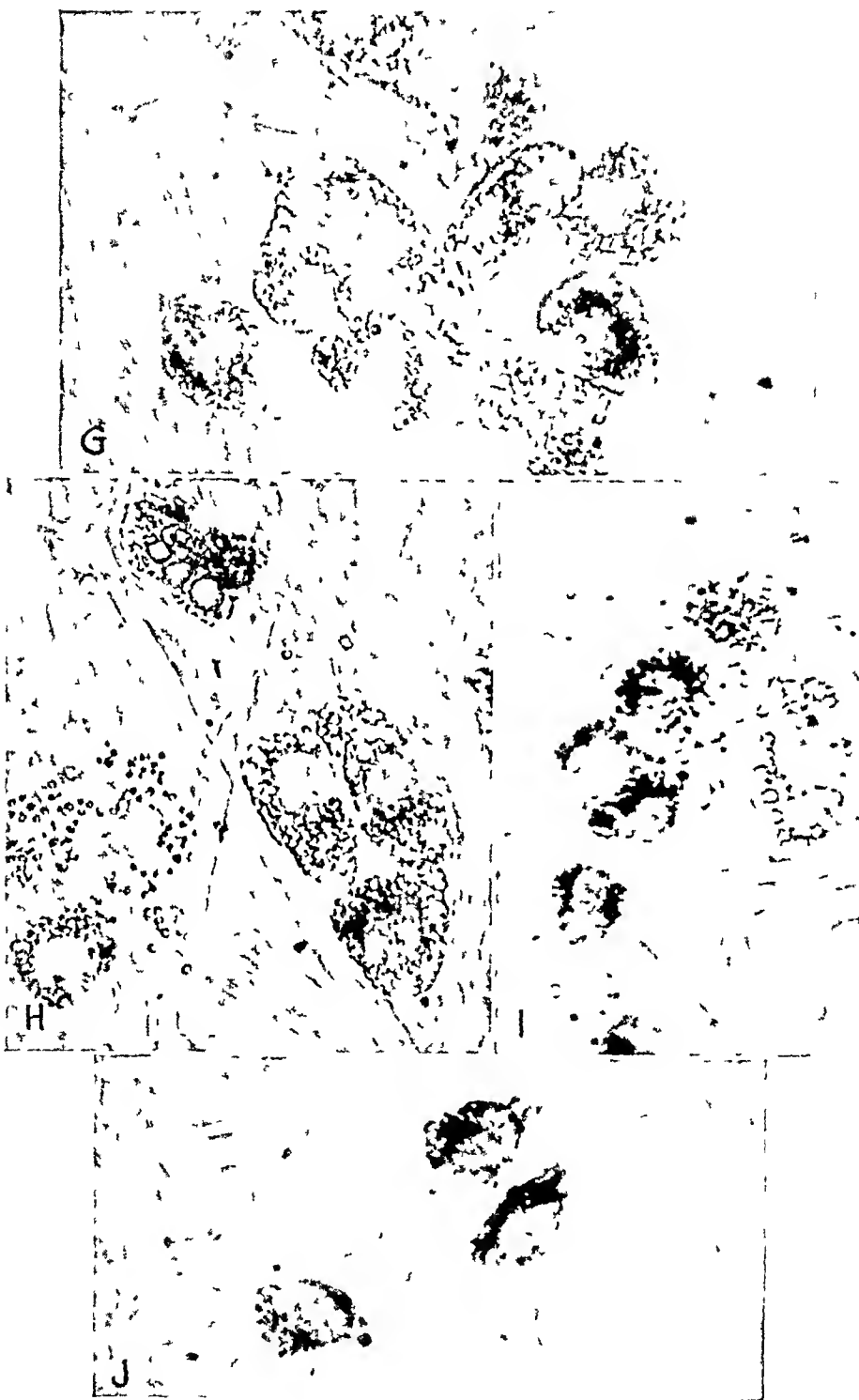


Figure 2
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While observations were continued much longer, the foregoing description seems to represent the sequence of events consecutively from start to termination following a single injection of cholesterol in colloidal suspension. Cytologic death occurred wherever cells were massed into closely packed foci, and this was followed by chemical reorganization with deposition of large plaques and needle-like crystals, together with the fats from cellular autolysis. The sequence of events following a single injection of cholesterol in colloidal suspension was subject to constant repetition in such areas but was modified by foreign body and fibrous reactions to the constituents of the foci. The cells in the centers of the foci were typical of the macrophages observed about forty-eight hours after the experimental injections. They were swollen with large anisotropic drops, which contained crosses of polarization and stained faintly with sudan. They were typical of the cells following injections of cholesterol in crystalline form described by earlier workers. They were obviously not end stages but intermediate stages (of the sequence of events just described) in areas where autolysis and crystalline depositions prolonged the processes of absorption and reaction. All the stages leading to the morphologic alterations described at three, four and five days after the experimental injections could be found along the axes leading outward from the foci, and thence outward were to be found the stages toward regression and resumption of the normal.

EXPLANATION OF FIGURE 2

The introductory comments on the films shown in figure 1 apply here except that crystals were no longer present.

G Mouse 87 Abdomen. Sufficient cholesterol was injected seven days previously to prolong the reaction for photographic purposes. This reaction is characteristic of the experimental reactions at three to four days. Tissue clasmotocytes and macrophages had become indistinguishable and occurred in masses. They were characterized by accumulations of anisotropic drops of lipid at the periphery of the cytoplasm and by the extreme uniformity, smallness and dark hue of the deposits of neutral red. The latter were abundant and were located central to the drops of lipid. The lipid contained crosses of polarization under polarized light.

H Same as *G*.

I Mouse 88 Abdomen. A colloidal suspension of cholesterol was injected eight days previously. Macrophages were in various stages of regression. The one at the right still contained considerable lipid. The three middle ones had lost practically all lipid, but still contained many deposits of neutral red, which had the characteristics of those illustrated in *G* and *H*. The upper and lower cells were intermediate in character. They still contained some lipid.

J Mouse 88 Back. Cholesterol in colloidal suspension was injected ten days previously. These three macrophages had practically finished the stages of regression. The two upper ones contained almost no fat and much less neutral red than previously. The lower one contained no fat. The fine, dark, uniform deposits of neutral red which were characteristic of the earlier periods had disappeared, and the deposits which were present were larger and more neutral in reaction than in the preceding periods. The cells were similar to normal tissue clasmotocytes.

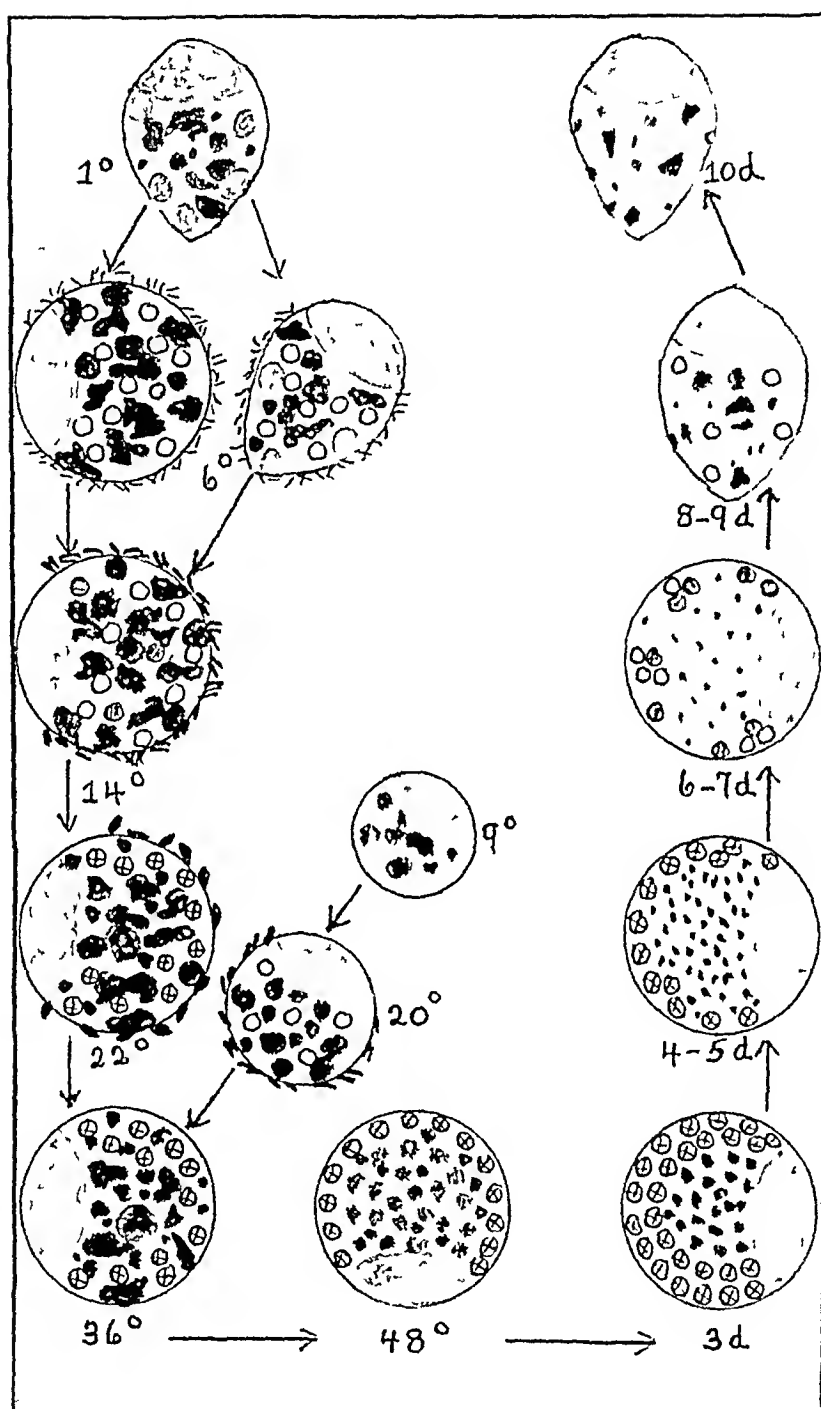


Fig 3—Schematic representation of the sequence of events observed in reticuloendothelial cells after cholesterol in colloidal suspension had been injected subcutaneously. The stated numbers of hours (1°, 6° and so on) and days (d) are merely indicative of the intervals after injection at which cells with any specific morphologic character began to occur with relative frequency. Comments on the character of the staining with sudan IV are added for comparison, but the staining with sudan IV is not represented.

(Legend continued on opposite page)

EXPLANATION OF PLATE

Solid gray or black areas represent deposits of neutral red of varying depths of color

Empty circles represent drops of isotropic fat

Cross-hatched circles represent drops of anisotropic lipid without crosses of polarization

Circles with crosses represent drops of anisotropic lipid with crosses of polarization

1 hour The tissue clasmatoocyte is slightly contracted. It contains neutral red vacuoles, "fluid vacuoles" and metachromatic vacuoles, but neither fat nor cholesterol (Sudan IV—diffuse stippling only)

6 hours Some tissue clasmatoocytes remain elongated, some have become rounded. The staining with neutral red is similar to that at one hour. Droplets of isotropic fat have become scattered at random in the cytoplasm, and many fine crystals of cholesterol are adherent to the surface (Sudan IV—diffuse stippling, also a few vacuoles rimmed with orange and a rare solid orange deposit)

9 hours Monocytes enter the field. They are small and contain neither fat nor crystals of cholesterol. The deposits of neutral red are small and neutral in reaction

14 hours The deposits of neutral red in the tissue clasmatoocytes have become larger, darker and more refractive than at six hours. Lipid droplets are scattered at random in the cytoplasm as at six hours, but some of them are now anisotropic. The crystals of cholesterol adherent to the surface tend to be arranged in parallel and are thicker than at six hours (Sudan IV—numerous areas that are either rimmed with orange or solid orange)

20 hours The monocytes have developed to macrophages. At this stage they contain much larger deposits of neutral red than at nine hours. This, however, is still neutral in reaction. Coarse acicular crystals of cholesterol adhere to the surface, and drops of isotropic fat are scattered at random throughout the cytoplasm

22 hours All of the lipid in the tissue clasmatoocytes is now anisotropic, and crosses of polarization are present. The crystals adherent to the surface are much fewer and much coarser than at fourteen hours. They have the shape of grains of rice. The deposits of neutral red are the same as at fourteen hours. Cellular debris has been phagocytosed (Sudan IV—numerous large, solid orange deposits)

36 hours The tissue clasmatoocytes and the metamorphosing macrophages have become indistinguishable. Crystals of cholesterol have disappeared from the surfaces of both. Both contain anisotropic drops of lipid which exhibit crosses of polarization and are tending to be arranged at the periphery of the cytoplasm. The deposits of neutral red are dark and refractive in both. They are becoming more central in location and slightly smaller (Sudan IV—more solid orange deposits than at twenty-two hours)

48 hours The drops of anisotropic lipid with crosses of polarization are now practically entirely peripheral in location. The deposits of neutral red are much smaller than at thirty-six hours, more uniform and more centrally located (Sudan IV—great numbers of large solid orange deposits)

3 days The accumulation of anisotropic drops with crosses of polarization is at the maximum. The drops are packed thickly at the periphery of the cytoplasm. The deposits of neutral red are packed more centrally. They are abundant and smaller than at forty-eight hours. They are still dark and refractive (Sudan IV—maximum number of large solid orange deposits. These tend to have a peripheral location. The cytoplasm central to them is very basophilic)

4-5 days The anisotropic drops at the periphery of the cytoplasm have begun to disappear. The deposits of neutral red central to them remain abundant and are even smaller than at three days (Sudan IV—fewer and more scattered solid orange deposits than at three days)

6-7 days The drops of lipid have almost disappeared. Many are no longer anisotropic. The deposits of neutral red are now disappearing also. They are the same size as at four to five days (Sudan IV—scattered solid orange deposits mingled with vacuoles which are merely rimmed with orange)

8-9 days Very few drops of lipid are now present. They are all isotropic. The fine deposits of neutral red have disappeared. The deposits present are now large, irregular in shape, neutral in reaction or buff in color, and less refractive than formerly. The cells are more like normal tissue clasmatoocytes than at any time since the injections (Sudan IV—merely a few vacuoles rimmed with orange)

10 days The cells are elongated and do not contain lipids. They contain little neutral red, and that is characteristic of the deposits in normal tissue clasmatoocytes (Sudan IV—diffuse stippling only)

COMMENT

Technics—The limitations and the specifications of the different technics for staining lipids are discussed in various texts on the subject and have been critically analyzed by Lison⁸. Discussion of the technics employed in these experiments seems indicated, therefore, only so far as they apply to the deductions to be drawn from the data presented.

Since all the lipid studies were made either from living or from air-fixed films, the lipids were in as nearly normal antemortem state as possible. The probability of their having been chemically changed dissolved by fixatives or otherwise modified, which usually has to be taken into consideration in histologic studies of lipids, seems unlikely in these studies.

The supravital preparations permitted a surprising degree of analysis and demonstrated the possibilities inherent in this method for the study of lipids. The technic served in one respect in which the other technics failed entirely. It permitted detection of the tiny acicular crystals of cholesterol which were free in the field and adherent to surfaces of the tissue clasmatocytes and macrophages up to thirty-six hours after the injections. The crystals did not stain by any technic. They were so small and their refractive index was such that they were not discernible in the mounts of glycerin jelly, nor was the space occupied by them usually demonstrable after mounting in balsam. On the other hand, they were strikingly evident when observed supravitaly by their refractivity. They were too delicate to be definable as separate crystals by either the Schultz or the digitonin technics.

The brilliant refractivity of the unstained drops of lipid in the supravital preparations made them, also, demonstrable at a smaller size and at earlier periods than was possible with any of the fixation technics employed. This was particularly striking in the studies of fibroblasts degenerating polymorphonuclear cells and young macrophages. Single droplets were often brilliantly refractive in the supravitaly stained films at early hours after injection, when the companion fixed films had not yet begun to stain by any of the other technics.

Not only were the lipid droplets demonstrable in the living films, but they could be clearly separated into isotropic and anisotropic lipids at either body or room temperature. If isotropic, the droplets obviously consisted of either fatty acids or triglycerides⁸. The fact that blue deposits were absent after staining with Nile blue sulfate throughout the entire experiment seems to rule out the possibility of free fatty acids. When the droplets became anisotropic, they obviously contained other lipids. Demonstration of the cross of polarization was facilitated

8 Lison, L. Bull d'histol appliq a la physiol 10 237, 1933

by the absence of fixation⁸ The phenomenon, when present, delineated the lipid into esters of cholesterol, vs free cholesterol, or into phospholipids or glycolipids

The Schultz technic is destructive of cytologic detail and therefore permits detection of the cholesterol radical merely grossly, rather than as specific deposits within cells It does not permit differentiation between cholesterol and its esters However, most contributions concerning the histochemical detection of cholesterol and its esters assume that a cross of polarization occurring in the presence of this radical indicates the presence of the esters rather than of free cholesterol The truth of this assumption seems to have been satisfactorily determined by Adam and Ashoff⁹ In these experiments, therefore, the Schultz technic served to demonstrate cholesterol-containing material within the cells, while the cross of polarization indicated that it was in ester form

These deductions were corroborated by the studies with digitonin That technic proved to be serviceable only when cholesterol or its esters were present in sufficient amounts for detection Formation of large enough particles of the sterol-digitonide for certain detection under polarized light and separation from esters was possible only after the colloidal particles of cholesterol had aggregated or after the cells became loaded with esters At the early hours after the injections, when the colloidal particles were free or adherent to the tissue clasmatocytes, the complex was rarely discernible In the later hours, however, the films exposed to digitonin served to demonstrate that cholesterol surrounded the cells and that esters filled them At still later periods, *i e*, at four or more days, the films served to demonstrate that both esters and cholesterol had been secondarily deposited as large masses in the foci of packed cells

Detection of the intracellular deposits of lipid by sudan IV was more sensitive than that by any of the other technics except study of the living films The heavily loaded cells in the centers of the packed foci stained only slightly, but elsewhere the cells stained deeply, the former obviously must have consisted largely of esters of cholesterol, while the latter contained generous components of neutral fats The tinting of the intracellular deposits with osmic acid indicates that oleic or other unsaturated fatty acids were present in moderate amount either in the component of neutral fat or as an ester of cholesterol

In summary, then, the supravital preparations served to demonstrate the presence and the time of disappearance of acicular crystals of cholesterol, both free and on the cell surfaces They served to demonstrate the presence of isotropic lipids, *i e*, neutral fats or fatty acids, in the cells before the anisotropic lipids, *i e*, cholesterol esters, became demonstrable and before reaction to lipid stains became positive

⁹ Adam, J G, and Ashoff, L Proc Roy Soc, London, s B 78 359, 1906

Finally, they served to demonstrate anisotropism and crosses of polarization with greater surety than was possible in fixed films. Histochemical technics with fixed films were necessary to demonstrate the presence of the cholesterol radical in the lipid vacuoles versus that of other anisotropic lipids. The occurrence of the cross of polarization indicated that this was in ester form.

Analysis of the Deductions from the Data Concerning Lipids—If the foregoing analysis of technics may be accepted, the sequence of events following the presentation of colloidal suspensions of cholesterol to the tissues seems to have been as follows. The scattered chylomicron-like globules of cholesterol in the suspensions were deposited as discrete fine acicular crystals as soon as the menstruum was absorbed (a matter of about three hours), the crystals were electively attracted to the surfaces of any tissue clasmatocytes in the area and to those of the macrophages which entered later. Isotropic fat appeared in the clasmatocytes and the macrophages slightly before anisotropic lipid was demonstrable. The crystals gradually increased in size even as they were gradually disappearing. They grew to the shape of grains of rice before all had disappeared. Disappearance seemed to take place mostly on the cell surfaces, although it cannot be stated that free crystals did not disappear without adhering to cells. As the crystals disappeared, the drops of lipid within the cells increased, became anisotropic and acquired crosses of polarization. According to the preceding discussion of technics, the crystals of cholesterol were converted to esters and entered the cells as such. All crystals had disappeared within thirty-six hours. The anisotropic drops of cholesterol esters acquired a peripheral position within the cells and continued to increase in number up to seventy-two hours. They gradually decreased thereafter. Few refractive dioplets of lipid were present within the macrophages by six days after the injections, and, of these, most were no longer anisotropic.

Whether esterification of the colloidal cholesterol occurs only on the surfaces of clasmatocytes and macrophages, or in the intercellular fluid also, is not definite, but seems likely. Shope,¹⁰ Sperry¹¹ and Schonheimer and Yuasa¹² have demonstrated an enzyme capable of esterifying cholesterol in connective tissues, but the technics which they employed do not permit conclusion as to whether it is intracellular or extracellular in location. The fact that both fibroblasts and polymorphonuclear cells in the area acquired dioplets of neutral fat and yet at no time acquired anisotropic droplets, i. e., cholesterol esters, would lead to the opinion that either these cells, in contrast to tissue

10 Shope, R. E. J. Biol. Chem. **80** 127, 1928

11 Sperry, W. M. J. Biol. Chem. **113** 599, 1936

12 Schonheimer, R., and Yuasa, D. Ztschr. f. physiol. Chem. **180** 19, 1929

clasmatocytes and macrophages, are impermeable to cholesterol esters in their environment or that the esters were formed only at the surface of the reticuloendothelial cells by virtue of esterase within these cells. The latter explanation seems the more likely. Correlation of these observations lends probability to the concept that while the presence of neutral fats is necessary if cholesterol is to be converted to its esters, the process of conversion takes place on the surfaces of cells of the reticuloendothelial system and not in the tissue spaces.

Correlation of the Sequence of Events in the Lipid Vacuoles and the Sequence of Changes in the Cytoplasmic Elements Which Stained with Neutral Red—The terms "neutral red vacuoles," "phagocytic vacuoles," "digestive vacuoles," "excretory vacuoles" and "supravital vacuoles" have been used rather indiscriminately and synonymously in most discussions concerned with the supravital technic. The present studies with cholesterol emphasize that the areas in phagocytic cells which stain with neutral red may differ considerably from the areas in which materials are first phagocytosed and deposited within the cytoplasm. In other words, the truly phagocytic vacuoles and the neutral red vacuoles may, or may not, be independent structures. In these studies the vacuoles which contained esters of cholesterol did not stain with neutral red. They can obviously be regarded as phagocytic vacuoles. On the other hand, the elements which stained with neutral red went through metamorphoses as striking as those of the lipid vacuoles and characteristically correlated with them in time. It would seem, therefore, that the areas stained with neutral red may be regarded as the truly digestive and excretory areas. In support of this concept is the fact that these areas were not demonstrable by the technics for lipids, did not vacuolate after exposure to fat solvents and stained simply as a part of the general cytoplasm in the fixed films. It would seem, therefore, that they represent specialized cytoplasmic elements with the chemical reactions of cytoplasm, i. e., of proteins, but capable of specific supravital staining.

The neutral red deposits of the tissue clasmatocytes changed characteristically soon after the injections. The same was true of the deposits in the macrophages as soon as those cells developed. The deposits increased greatly in size and number, became darker red, i. e., more acid, and more refractive than normal, and the cells containing them became swollen and rounded. These changes were more or less synchronous with the appearance of isotropic droplets within the cells and preceded the appearance of anisotropic material. They continued, however, after onset of the latter event. As the free crystals of the cholesterol decreased in number on the surfaces of the cells and the anisotropic drops containing the cross of polarization increased within the cells, the elements stained with neutral red gradually

decreased in size, even while they increased in number in the process. They remained dark in color. They thus became strikingly uniform in both color and size. At the same time they became centrally located in the cells, and arranged as a large rosette in the area of the centrosome, which was framed by the anisotropic drops of cholesterol ester at the periphery of the cytoplasm. These changes in the deposits of neutral red began as early as thirty-six hours after the injections. The fineness and the uniformity of the deposits were striking by seventy-two hours, the period at which the greatest number of anisotropic drops occurred. The deposits remained abundant but continued to decrease in size during the next two days, when the anisotropic drops were decreasing in number. At this time the cells gave every indication of becoming epithelioid in type. Decreases in the size of the neutral red deposits, however, did not progress beyond that attained on the fourth and fifth days after the injections. The deposits then gradually disappeared without attaining the dustlike fineness of those in epithelioid cells and without the development of binucleated cells or other evidences of cellular degeneration. In this respect the cellular reaction was similar to the response which Gray² observed in guinea pigs after injections of tributyrin. Disappearance of the deposits of neutral red followed on the disappearance of the droplets of cholesterol ester. By eight days any deposits of neutral red had become scattered, larger in size, lighter in color, angular in shape and much like the deposits in normal tissue clasmatocytes.

Correlation of the changes in the neutral red deposits with the changes in the droplets of cholesterol ester suggests the possibility that the former are directly related to the latter and represent end stages in a series of events beginning at the cell surface and progressing toward the completed stage represented by the special cytoplasmic elements which stained with neutral red in the area of the centrosome. The fact that the peak of events in the deposits which stained with neutral red occurred later, and ended later, than the peak of events in the lipid droplets lends support to this concept. If this concept is correct, one may conceive that the neutral red deposits in these experiments were end products of the digestion of the cholesterol esters, and of such nature that they had lost the ability to react to fat stains or to be dissolved from the cytoplasm by the usual fat solvents. That is to say, they behaved in the manner of the lipoprotein elements of cells, which, in this case, would seem to be represented by one of the combinations of protein and cholesterol. A discussion of these has recently been presented by Bloor¹³.

13 Bloor, W. R. *Biochemistry of the Fatty Acids and Their Compounds, the Lipids*, American Chemical Society Monograph Series, New York, Reinhold Publishing Corporation, 1943.

The reticuloendothelial cells are obviously able to attract cholesterol that is in a colloidal state and probably are able to convert it into esters. They are certainly able to absorb it as esters. Further, having absorbed the esters, they are able to handle them so that they ultimately disappear without injury to the cells unless the cells themselves are handicapped by too great a degree of crowding. If the theory presented in this study is tenable, they are converted into combinations of cholesterol and protein within the individual cells, and excreted as such.

Literature—The findings about the foci of necrotic masses of cells at considerable periods after injections of the colloidal suspensions of cholesterol, and the findings after injections of concentrated emulsions with more or less aggregation, as well as the findings of earlier workers who employed crystalline cholesterol suspensions of varying strengths, are in contrast to the primary reactions observed with colloidal cholesterol suspensions as they have been presented. In the former instances the coarse needle-like or plaquelike crystals deposited were surrounded by giant cells as well as by mononuclear cells similar to the macrophages which occurred three or four days after the injections of the colloidal suspensions. These reactions were intermingled with necrosis and proliferation of fibrous tissue. Responses of this type represent a mixture of reactions. One is the foreign body reaction, or the reaction to large insoluble material. The other is a reaction essentially like that described after the injections of cholesterol in colloidal suspension. It is represented by the large cells filled with anisotropic drops which were long recognized as cholesterol esters. That these cells were continually present at late periods rather than in the regressive stages described in the present experiments seems to be due to the fact that cholesterol was being constantly presented to them as the large crystals were slowly dissolved in the fats liberated in the processes of necrosis. The reaction may be considered to have been repeating itself indefinitely until all of the crystals could be dissolved and absorbed by the cells. Necrosis apparently took place in the process, however, and sufficient neutral fat and fatty acids were liberated to stimulate proliferation of fibrous tissue. The early workers in this field examined the reaction only after it had reached this stage (Basten,¹⁴ LeCount,¹⁵ Schonheimer and Yuasa,¹² Reiss¹⁶ and Kimmelstiel and Laas¹⁷). It is only recently that the differences in the reaction to cholesterol in the colloidal and in the crystalline state are beginning to

14 Basten, G. *Virchows Arch f path Anat* **220** 176, 1915

15 Le Count, E. R. *J M Research* **7** 166, 1902

16 Reiss, H. *Virchows Arch f path Anat* **296** 627, 1936

17 Kimmelstiel, P., and Laas, E. *Beitr z path Anat u z allg Path* **93** 417, 1934

be appreciated Both Hirsch¹⁸ and Weinhouse¹⁹ have suggested that the reaction to cholesterol in colloidal form is the more dynamic reaction of the body, and of very different import from the reaction to cholesterol in crystalline form

These studies, then, lead to the conclusion that the cells of the reticuloendothelial system are specifically constituted to handle cholesterol They react to it in one way when it is presented in solid form, and in another when it is presented in colloidal form The latter seems to represent the specific, direct reaction by which cholesterol is absorbed from the tissues, modified, combined and excreted back into the body fluids The reticuloendothelial cells in the subcutaneous tissue that take part in this reaction are from two sources, i e, the preexistent tissue clasmatocytes and the macrophages that develop from monocytes Both types of cells react the same and eventually become indistinguishable Both electively attract the acicular crystals of cholesterol The cholesterol is converted into esters at the cell surfaces The esters are absorbed and segregated as liquid crystals in vacuoles at the periphery of the cytoplasm The elements which stain with neutral red and which are more centrally located in the cytoplasm pass through a series of transitions which suggest conversion of the esters into combinations of cholesterol and protein and their ultimate excretion in that form After completion of the stages outlined, with disappearance of the fine deposits of neutral red that have been evolved in the process, the cells regress to a morphologic state which is like that of normal tissue clasmatocytes and decrease in number The time required for this complete cycle is considerable, and undue crowding of the reactive cells may result in sufficient interference with nutrition and absorption to cause them to succumb The reactions which follow in that event are no longer characteristic of the response to cholesterol alone

CONCLUSIONS AND A HYPOTHESIS

Cholesterol in colloidal suspension is deposited subcutaneously as discrete acicular crystals which are electively attracted to the surfaces of cells of the reticuloendothelial system These are represented by both preexisting tissue clasmatocytes and macrophages which develop from hematogenous cells

The acicular crystals are converted to cholesterol esters and enter the cells It seems, but is not definitely proved, that this process occurs only at the cell surfaces, by virtue of esterase within the cells, and not in the interspaces

18 Hirsch, E F Arch Path **25** 35, 1938 Hirsch, E F, and Weinhouse, S *ibid* **30** 1097, 1940

19 Weinhouse, S Arch Path **35** 438, 1943

The esters become segregated in the peripheral part of the cytoplasm as liquid crystals. They gradually disappear, their disappearance being concomitant with characteristic changes in the elements which stain with neutral red.

The elements stained with neutral red first enlarge and become refractive and acid in reaction. They assume central location as the esters accumulate in the peripheral part of the cytoplasm, decrease in size, increase in number and remain acid in reaction. These changes are followed by rapid dissolution and regression after the esters have disappeared.

The hypothesis is presented that the elements which stain with neutral red in these experiments represent combinations of cholesterol and cell proteins, and are excreted as such.

High concentrations of cholesterol or aggregations of cells crowded to the point of necrosis in areas reacting to a colloidal suspension of cholesterol result in crystalline depositions. In that case foreign body and fibrotic reactions merge with a continued response to the cholesterol in colloidal form.

SYSTEMIC MULTICENTRIC LIPOBLASTOSIS

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NO GROUP of tumors has more successfully resisted attempts at orderly arrangement than those of the lipid system. The pertinent problems are the same as those which obscure the embryogenesis of normal fat tissue, further difficulties arise from the frequent overlapping of morphologic aspects in the great variety of conditions in which overgrowth of fat tissue occurs. For these reasons the attempt to establish the nature of an unusual case of multiple recurrent fat tissue growths leads to the consideration of various related pathologic conditions,

SURVEY OF LITERATURE

The distinction generally drawn between obesity, multiple lipoma and lipomatosis in its several varieties—"symmetric diffuse," "dolorosa," "discrete" and "cervical" (Mosny and Beaufumé¹ and Madelung²)—does not always appear to be justified on morphologic grounds. Wells³ cited cases of multiple lipoma accompanied with tenderness such as is found in adiposis dolorosa (Dercum's disease). He also recalled cases classified as instances of sex infantilism with obesity (adiposogenital dystrophy, or Frohlich's syndrome) in which later the condition merged into generalized obesity or diffuse lipomatosis. The case observed by Vallery-Radot and associates⁴ of a young woman who displayed in succession a circumscribed lipoma of the thigh, then obesity and subsequently huge fatty nodules in the subcutaneous tissue further exemplifies the ill defined limits among 'lipopathies'.

Symmers and Fraser⁵ described a condition occurring in marantic infants which was attended by hyperplasia of the so-called primitive fat organs so pronounced as to resemble a neoplastic growth. They also recognized a group of chronic productive inflammatory conditions accompanied with hyperplasia of embryonal fat cells which infiltrate the

1 Mosny and Beaufume. Bull et mem Soc med d hop de Paris **19** 106, 1902

2 Madelung, O W. Arch f klin Chir **37** 106, 1888

3 Wells, H G. J A M A **114** 2177, 1940

4 Vallery-Radot, P. Blamoutier, P, and Krief, J. Bull et mem Soc med d hôp de Paris **48** 1083-1086, 1924, quoted by Coormaghtigh²¹

5 Symmers, D, and Fraser, A. Arch Int Med **19** 699, 1917

surrounding tissue in a neoplastic fashion. Their observations remind one of the so-called intestinal lipodystrophy (lipophagia granulomatosa), in which lipids are deposited in the mucosa of the small intestine and lipid-laden mononuclear cells are present in the mesenteric lymph nodes, simulating cancerous lipoblastoma.⁶

In addition, there are reports in the literature of diffuse lipomatosis which became locally progressive and exhibited wild potentialities of growth (Robertson⁷). The opposite course was recorded by Mirolli⁸ in his report of a case of long-standing diffuse lipomatosis, in which he observed gradual regression of the newly formed masses of fat tissue.

In spite of its definitely neoplastic characteristics, even a mature lipoma presents an intimate structure which does not differ basically from that of a nonencapsulated fatty overgrowth. Wells⁹ has emphasized that the mature lipoma and the steatopygous deposits of the Hottentot women arise in the same way, from embryonic mesenchymal cells of the preadipose tissue; this same histogenesis has been advocated for the so-called heterologous lipoma (Chiari⁹) and also applied to the fatty nodules occasionally encountered in the subcutaneous adipose tissue, often mistaken for lipoma, and defined by Mallory¹⁰ as "non-encapsulated moruloid tumors of the adult adipose tissue."

Immature cells appearing in a fatty growth may lead to the false conception of a cancerous process (Jaffe¹¹). On the other hand, an entirely mature lipoma, arousing no untoward suspicion from the histologic standpoint, may be the site of repeated recurrences. Ewing¹² reported a case in which a lipoma of the spermatic cord recurred many times in the course of fifteen years in spite of the consistently mature appearance of the fat cells on repeated histologic examinations. A retroperitoneal lipoma described by Horn¹³ displayed a similar behavior, and identical observations by Winkler,¹⁴ Hosemann,¹⁵ Lang,¹⁶ Labey,¹⁷

6 Whipple, G. H. *Bull. Johns Hopkins Hosp.* **18**: 382, 1907. Blumgart, H. L. *Arch. Int. Med.* **32**: 113, 1923. Jarco, S. *Bull. Johns Hopkins Hosp.* **59**: 275, 1936. Reinhart, H. L., and Wilson, S. J. *Am. J. Path.* **15**: 483, 1939. Sailer, S., and McGann, R. J. *Am. J. Digest. Dis.* **9**: 55, 1942. Hill, J. M. *Am. J. Path.* **13**: 267, 1937. Pearse, H. E. *Surgery* **11**: 906, 1942. Fitzgerald, P. J., and Kinney, T. D. *Am. J. Path.* **21**: 1069, 1945. Amsterdam, H. J., and Grayzel, D. M. *Am. J. M. Sc.* **210**: 605, 1945.

7 Robertson, H. E. *J. M. Research* **35**: 131, 1916.

8 Mirolli, A. *Riforma med.* **44**: 1624, 1928.

9 Chiari, H. *Tr. Chicago Path. Soc.* **8**: 65, 1910.

10 Mallory, F. B. *The Principles of Pathologic Histology*, Philadelphia, W. B. Saunders Company, 1914.

11 Jaffé, R. H. *Arch. Path.* **1**: 381, 1926.

12 Ewing, J. *Neoplastic Diseases*, ed. 2, Philadelphia, W. B. Saunders Company, 1922.

13 Horn, cited by von Wahlendorf³⁴.

Williams¹⁸ and many others seem to justify the conclusion of Seids and McGinnis¹⁹ that such growths should be considered as cancerous regardless of their morphologic aspect. In 4 of 5 cases of Seids and McGinnis in which there were recurrences, mature fat cells prevailed in the growth, the concomitant presence of spindle-shaped or rounded, rather anaplastic cells might, however, be considered as an evidence of a potential cancerous tendency.

In reporting 2 cases of recurrent lipomatous growth of the groin, Jaffé¹¹ discussed the concept of cancer in lipoblastoma. Sarcomatous change within the stroma of a lipoma does not fulfil in his opinion the qualifications of "liposarcoma." From the group of tumors diagnosed as liposarcoma he also excluded the mixed tumors in which fat tissue is associated with other types of tissue exhibiting evidence of cancer but in whose growth the fat tissue per se seems to play a passive role. He also doubted the sarcomatous nature of the capsulated lipoblastoma which, although composed of embryonic fat cells (lipoblasts), shows little evidence of cellular anaplasia and in which, as a rule, mitotic figures are absent. The term "liposarcoma" is reserved, accordingly, for the growths showing anarchy of fat storage cells, frequent recurrences, invasive power and high metastasizing tendency.

Tumors classified as liposarcoma, in the strict meaning of the term are in turn subdivided by Ewing²⁰ into two varieties: an adult fat cell type, composed of granular cells simulating closely those found in chronic inflammation of fat tissue, and an embryonal fat cell type, characterized by incomplete differentiation of perivascular mesenchymal cells which, instead of developing into mature fat cells, stop in early phases of their maturation and exhibit either myxomatous or foamlike properties. Transformation from one cytologic type to another is not rare. Lang¹⁶ has reported a case of primary retroperitoneal lipomyxoblastoma which on recurrence showed the patterns of a mature lipoma to revert to the original lipomyxomatous structure on second recurrence.

From the standpoint of localization, conditions diagnosed as lipoblastoma and lipoblastosis, respectively, are divided by Goormaghtigh and colleagues²¹ into two groups, the ones arising in the subcutaneous tissue and the ones arising in the internal cavities and deep organs. Lipoblastosis bears to lipoblastoma, according to them, the same relation

14 Winkler, A. *Ergebn d allg Path u path Anat* **23** 22, 1930

15 Hosemann, G. *Arch f klin Chir* **155** 336, 1929

16 Lang, W. *Arch f klin Chir* **155** 349, 1929

17 Labey. *Presse med* **42** 1775, 1934

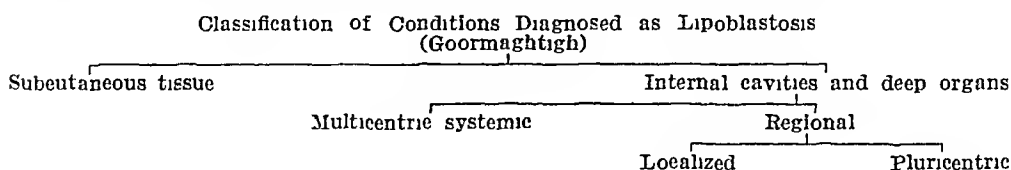
18 Williams, C. *J A M A* **105** 195, 1935

19 Seids, J V, and McGinnis, R S. *Surg, Gynec & Obst* **44** 232, 1927

20 Ewing, J. *Arch Surg* **31** 507, 1935

21 Goormaghtigh, N, Vanderlinden, P, and de Puyssseleyr, R. *Cancer, Bruxelles* **13** 3, 1936-1937

that diffuse fibromatosis holds to fibroma. Among the conditions diagnosed as lipoblastosis of the internal cavities, the authors distinguish fat tissue proliferations in a disorderly multicentric distribution affecting different parts of the body (multicentric systemic lipoblastosis) and fat tissue proliferations, either single or multiple, strictly limited to one region of the body (regional, localized or pluricentric lipoblastosis)



The retroperitoneal space is listed as the most frequent site of the localized regional growths, the perirenal region being the oftenest affected. The intermuscular spaces come second in order of frequency. The tendency of the new growths with intermuscular localization to follow the course of the peripheral nerve trunks has made some consider the possibility that there is an anlage of preadipose tissue in the nerve sheaths.²² The observations of Alsberg,²³ of multiple lipoma and neurofibroma in the same person, and that of Leven,²⁴ in which the two processes were seen concomitantly in two generations of one family, seem to support this contention. Adair, Pack and Farrior²⁵ went so far as to consider multiple lipoma as neurolipoma.

Bony localization is third most frequent, according to Goormaghtigh,²¹ who recognized benign lipoblastoma, composed of mature fat cells, as in the cases of Cornil and Ranvier,²⁶ and cancerous lipoblastoma, characterized by cellular atypism, radiosensitivity and tendency to metastasize early throughout the body, as in the cases of Stewart,²⁷ Barnard²⁸ and Fender.²⁹

The occurrence of localized fat tissue growths in the thoracic cavity is more rarely seen, it is illustrated in the case of Narr and Wells.³⁰

The abdominal cavity is still the leading site for the pluricentric regional growths. In the cases of Hirsch and Wells³¹ the sites of fat

22 Delachanal, J. Des tumeurs malignes du tissu cellulo-adipeux, Thesis, Lyon, no 22, 1910. Bland-Sutton, J. Tumours, Chicago, W. T. Keener & Co., 1907. Mesa, C. Prensa med argent **26** 1779, 1939.

23 Alsberg, A. Ueber Neurolipome, Inaug. dissert., Berlin, G. Schade, 1892.

24 Leven. Dermat Wchnschr **87** 1563, 1928.

25 Adair, F. E., Pack, G. T., and Farrior, J. H. Am J Cancer **16** 1104, 1930.

26 Cornil, V., and Ranvier, L. Manuel d'histologie pathologique Paris, F. Alcan, 1901, p. 393.

27 Stewart, F. W. Am J Path **7** 87, 1931.

28 Barnard, L. Arch Surg **29** 560, 1934.

29 Fender, F. A. Am J Path **9** 909, 1933.

30 Narr, F. C., and Wells, A. H. Am J Cancer **18** 912, 1933.

31 Hirsch, E. F., and Wells, H. G. Am J M Sc **159** 356, 1920.

tissue proliferation were the retroperitoneal space and the mesentery and other peritoneal folds, in Martland's case³² the pelvis, the retroperitoneal space, the perirenal region, the mesentery of the small intestine and the gastrocolic omentum were involved by the proliferative process. A diffuse anlage extending from the space of Retzius to the kidneys along the ureters in the retroperitoneal space might perhaps explain, as suggested by König,³³ the tendency of the tumors at this site to grow more rapidly than elsewhere and their cancerous potentialities. Among the 176 cases of retroperitoneal lipoblastoma analyzed by von Wahlen-dorf³⁴ there were 21 in which the growth had become cancerous, and among 115 in which the growth had been removed there were 15 in which it recurred in a short time.

Multicentric regional fatty growths are less frequently found in the thoracic cavity. In the case of Rokitsansky³⁵ the growths were located along the intercostal spaces. Two independent lipomatous masses composed of grapelike nodules of yellow fat tissue were present in the case of Klemperer and Rabin,³⁶ which they interpreted on the basis of a differentiation of embryonal mesenchymal cells into fat cells.

The multiple independent but strictly regional growths in the instances cited represent a connecting link between isolated lipoma and multicentric systemic lipoblastosis, with which the present study is concerned more directly. Only 6 cases of this type³⁷ have been found on review of the literature, their main clinical and structural peculiarities, together with those noted on personal observation, are summarized in the table. After the details of the present case have been given, its characteristics will be compared with those of the similar cases in the literature, in an attempt at unitarian interpretation.

REPORT OF A CASE

The study of this case was made possible by Dr. Paul Klemperer, of Mount Sinai Hospital, New York, who also provided the slides for the study of the manner in which fat tissue develops in embryonal and adult life.

F. C., a 34 year old white woman, was first seen in Mount Sinai Hospital, New York, in the summer of 1916. She complained of swelling in the right

32 Martland, H. *Arch Path* **5** 932, 1928.

33 König, cited by Goormaghtigh, and others²¹.

34 von Wahlen-dorf, A. L. *Arch f klin Chir* **115** 751, 1921.

35 Rokitsansky, cited by Goormaghtigh and others²¹.

36 Klemperer, P., and Rabin, C. B. *Arch Path* **11** 385, 1931.

37 (a) Nienhuis, J. H. *Ztschr f Krebsforsch* **22** 434, 1925. (b) Henke, F., and Lubarsch, O. *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1925, vol. 6, p. 692. (c) Siegmund, H. *Virchows Arch f path Anat* **293** 458, 1934. (d) Broca. *Bull et mem Soc anat de Paris* **25** 137, 1850. (e) Askanazy. *Virchows Arch f path Anat* **158** 407, 1898. (f) Goormaghtigh and others²¹.

popliteal space, of nine months' duration. Enucleation of the popliteal mass revealed a benign fat tissue tumor. Six years later, in 1922, the tumor recurred. In the meantime a large mass had appeared in the neck. Both the recurring popliteal mass and the new growth in the neck were removed. The pathologic diagnosis was still lipoma, prolific cellularity of the interstitial tissue suggested, however, the possibility that a cancerous change might have occurred. One year later, in 1923, a large lipomatous mass appeared on the right abdominal wall. It was removed in February 1925, together with another recurrent tumor of the right popliteal space. Symptoms of paraplegia appeared in the summer of 1927. The existence of a tumor of the spinal cord was suspected, and operation revealed an extradural fat tumor at the level of the eighth and ninth dorsal vertebrae. At this time evidence was found that the tumor of the neck had recurred. The symptoms of paraplegia, which had disappeared after the removal of the dural growth, reappeared in the fall of the same year. The old laminectomy wound was reopened, and a recurrent extradural fat tumor was enucleated. Numerous subcutaneous tumors developed in the meantime. In the following months the patient showed a gradual decline, and bilateral spastic paralysis with muscular atrophy and loss of sensation appeared in the lower extremities. A third laminectomy revealed recurrence of the old fatty tumor in the right side of the spinal dura. Death occurred twenty days later.

Postmortem Findings (summary of autopsy record) —Externally, the outstanding findings were a series of nodular swellings at both sides of the neck, a large mass bulging on the abdominal wall and a similar one in the left thigh, just beneath the Poupart ligament. The mass in the thigh was freely movable and oval in shape, measuring 13.5 by 12 cm. The right popliteal space was almost completely filled by a large, firm, resilient nodular mass, which measured 13.5 by 9 cm. In the back there was a laminectomy incision 14 cm long at the level of the thoracic portion of the spine, and deep in the wound a sausage-shaped mass was seen to surround the vertebral column from the fifth to the ninth dorsal vertebra and to penetrate the intervertebral foramina.

All these masses showed a similar appearance. They were soft in consistency and had homogeneous and smooth surfaces both externally and on the cut sections. The color was uniformly pale gray, in contrast to the orange-yellow of the surrounding healthy fat tissue. Fine bands of glistening, grayish pink tissue, crossing one another in network fashion, were noticeable here and there in most of the growths.

Other tumor masses, of identical appearance, were found in the internal cavities of the body. One, 3 cm in the largest dimension, was located beneath the seventh rib, another, about the same size, was embedded in the fat tissue at the bifurcation of the trachea. Three fatty nodules, each the size of a walnut, were present along the course of the left adrenal vessels, and another, apricot sized, was located in the mesentery of the ileum. The mesoappendix and the transverse mesocolon were infiltrated by similar nodular fat tissue growths. A large mass, 8 cm across, almost completely obliterated the cul-de-sac of Douglas.

Extensive sacral decubitus, with deep ulceration of the skin and severe inflammatory reaction, four stones in the cystic duct and a severe necrotizing pyelocystitis were additional postmortem findings.

Microscopic Observations—Comparative examination of the slides of the popliteal mass that developed first, the slides of the numerous tumor masses removed at subsequent operations and the slides of the masses found at autopsy twelve years later revealed no substantial structural differences. Review of the

Systemic Multicentric Lipoblastosis

| Case and Author | Clinical Course and Symptoms | Localization of the Fatty Growths | Microscope Features | Diagnosis | Other Significant Findings |
|--|---|---|--|--|---|
| 1 Man, aged 31 (Broca, quoted by Ewing) | After removal of a lipoma from thigh, hundreds of fat tissue tumors developed, growths remained stationary about 40 yr terminal symptoms of dysphagia | Subcutaneous tissue, mesentery, periesophageal region | Atrophic fat tissue interspersed by bands of fibrous connective tissue, numerous foci of lymphocyte like cells irregularly scattered | Multiple lipoma | Hypertostosis of skull |
| 2 Woman, aged 33 (Askaniy, 1899) | Patient died a few days after operation on the neck for suspected tumor of thyroid gland | Subcutaneous tissue, breasts, mesentery, axilla and liver | Transitional forms from undifferentiated mesenchymal cells to ripe fat cells, the latter prevailing | Lipoblastic sarcoma with metastasis | |
| 3 Man, aged 55 (Nienhuis, 1925) | Two yr duration spastic paraplegia and diabetes | Mesentery, retroperitoneal space, pancreas, mesogastrium, colon, pelvis mediastinum, thoracic vertebrae, sternum, femur, spinal and cerebral dura | Mature fat tissue with myxomatous areas, cellular zones within bone marrow fatty growths | Metastasizing lipoma,* the primary source believed to be capsule of kidney | Osteosclerosis of skull and hyperostosis in forehead, calcified cysticercus in right postcentral convolution, internal and external hydrocephalus |
| 4 Man, aged 49 (Lubarsch, 1925) | Three yr duration, huge subcutaneous lipomatous masses developed after removal of lipoma from left thigh | Subcutaneous tissue, mediastinum, myocardium, mesentery, serosa of large and small intestines, renal capsule, urinary bladder pelvis, vertebrae, femur and around abdominal aorta | Transitional forms from embryonal mesenchymal cells to ripe fat cells, fibrous and myxomatous areas | Lipoblastic sarcoma | |

| | | | | | |
|--|---|--|--|---|------------------------------------|
| 6 Man, aged 50 (Goormaghtigh, Vanderlinden and de Puysselcy, 1930) | Numerous subcutaneous lipomatous growths devel- oped throughout an unspec- ified period of years | Subcutaneous tissue, upper and lower extremities, right groin, apex of heart (750 Gm.), capsule of right kidney (850 Gm.), medulla of left kid- ney great and small epiploon, epiplole appendices, retro- peritoneal space, large intes- tine, mesentery, diaphragm 214 independent fatty growths altogether | Transitional forms from undifferentiated mesenchymal cells to ripe fat cells, with a scattering of small lympho- cyte like cells around numer- ous blood capillaries, fibrous and myxomatous areas | Systemic multicentric lipoblastosis | Acute disseminated tuberculosis |
| 7 Woman, aged 31 (Tedeschi) | Twelve yr duration, process started with popliteal fatty tumor diagnosed benign, 6 yr later recurrence and new fatty growth at neck, both removed and again consid- ered benign, 1 yr later new growth in abdominal wall and second recurrence of popliteal mass, 2 yr later development of extradural spinal fat tissue growth causing paraplegia, also first recurrence of growth in neck After 3 mo first recurrence of extradural spinal mass and development of numer- ous subcutaneous tissue tumors, death following re- moval of second extradural recurrence | Subcutaneous tissue, neck abdominal wall, thigh, pop- liteal space, extradural spinal space, thoracic cavity, adrenal region mesenteric, meso- appendix, transverse mesocolon pelvis | Transitional forms from embryonal mesenchymal cells to ripe fat cells with marked vascularity and foci of extra- medullary hemopoiesis | Systemic multicentric lipoblastosis | |

* This case was considered by both Siegmund and Goormaghtigh as diffuse lipoblastosis

sections from the primary popliteal mass showed it to be composed of mature fat cells, which were supported here and there by a thin interlacing of well vascularized fibrous connective tissue bundles, from which an arborizing capillary network branched into the bulk of the tumor. In between the fat cells there was a scattering of connective tissue cells, mostly fibroblasts and lymphocytes, irregularly mixed with younger mesenchymal cells which showed cytologic characteristics of undifferentiated cells. The latter were irregularly shaped, with occasional cytoplasmic processes, large nuclei, well defined nuclear membranes, scanty amounts of chromatin and one to three nucleoli. Within the cytoplasm of some of these cells were minute fat droplets, orange stained in the sudan III sections, and where the fat droplets were larger and more numerous, the cells assumed, through swelling of the cellular body and withdrawing of the cytoplasmic processes, a polygonal or rounded shape, while the nucleus appeared displaced toward the periphery.

As stated, the sections of the tumor masses removed at subsequent surgical interventions displayed no difference when compared with the primary popliteal growth, and fundamentally identical patterns were shown by the sections from the postmortem material. In the latter most of the growths still consisted of apparently normal fat tissue, but the fat cells varied greatly in size, the supporting stroma was irregular in distribution and there was a distinct increase in cellularity. Among the non-fat-bearing cells, young mesenchymal cells prevailed over less numerous fibroblasts and lymphocytes. These young mesenchymal cells either were sparsely scattered in between the mature fat cells or had coalesced into small nodular formations which, owing to the presence of immature hematic cells (myelocytes and nucleated red blood cells), assumed the appearance of foci of extramedullary hemopoiesis. Fat deposits were seen within the young mesenchymal cells oftener in the sections from postmortem material than in the sections from the surgical specimens. The evidence of cytoplasmic fat storage ranged from minute granule-like droplets to large lipid vacuoles, which on fusion resulted in an adult fat cell with a single large vacuole or several smaller ones bounded by a thin cytoplasmic ring. The nucleus which had been centrally placed in the early stages of cellular differentiation, appeared sickle shaped, flattened and compressed at the periphery in the matured cell. Even in the most cellular zones there was no evidence of cellular anarchy and no mitotic figures could be seen.

The blood capillaries were abundant and frequently appeared surrounded by thin mantles of young mesenchymal cells. In these vascular areas transitional forms were distinctly observed, ranging from undifferentiated mesenchymal cells to maturing fat cells.

Regressive changes were present here and there and consisted mainly in a pseudomucinous degeneration, as shown by large blocks of eosinophilic structureless material spreading apart and at times overshadowing the proliferated fat tissue cells.

COMMENT ON CASE AND COMPARISON WITH PREVIOUS CASES

Multiple, recurrent nonencapsulated fat tissue growth, involving in a scattered fashion both the subcutaneous tissue and the internal cavities of the body, was the unusual characteristic in the case here reported, for which, accordingly, the appropriate label seems to be "systemic multicentric lipoblastosis."

The process started in the popliteal space as an apparently benign lipoma, which was removed, the multicentric proliferation of fat tissue began six years later, and, in spite of numerous attempts to control the growths by repeated surgical intervention, a series of recurrences brought the patient to death in the course of twelve years. Among the various localizations the one in the extradural spinal space deserves special consideration, because it must be regarded as the direct cause of death and because of its extreme rarity. Stookey,³⁸ in reviewing the literature up to 1928, was able to find only 1 case of extradural lipoma, and Elsberg,³⁹ among 179 extradural spinal tumors, listed a single lipomatous growth, the one described here. Of the six reports of cases of systemic multicentric lipoblastosis summarized in the table, dural localization is mentioned in only one, that by Nienhuis.^{37a}

In trying to explain the multiple fat tissue growths, several questions arise: (1) whether the lipomatous masses are the exponent of a widespread process metastasizing from a primary cancerous lipoblastoma or whether they are autonomous and independently originated, and (2) if they should be considered independent, whether they are neoplastic in nature, in the strict meaning of the term, or are the expression of a systemic developmental or dyscrasic disturbance of the lipid system resulting in multiple autonomous growths.

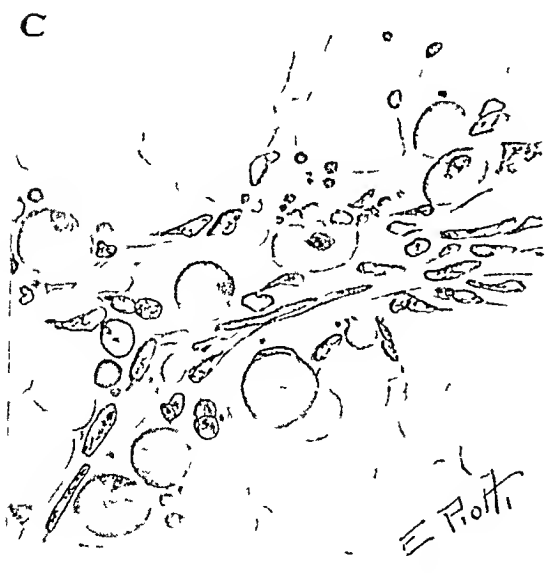
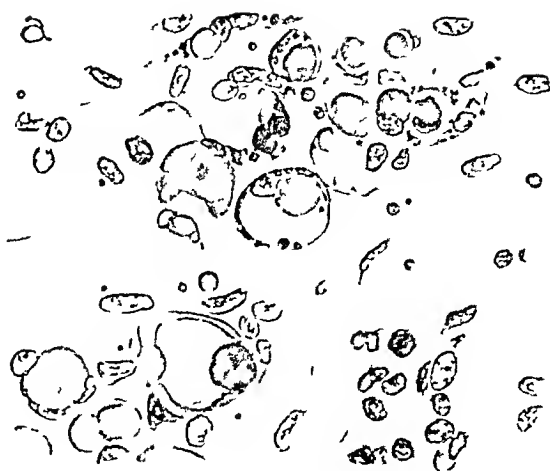
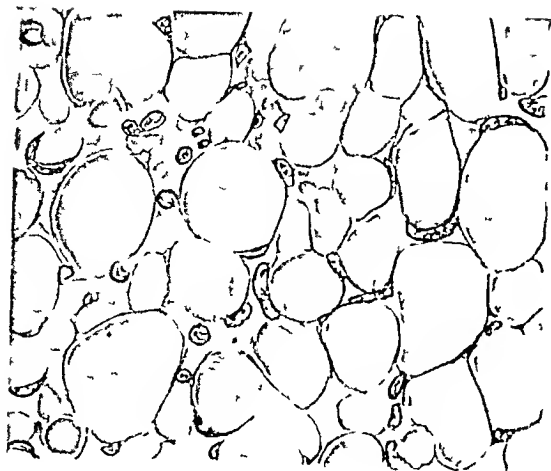
Nienhuis^{37a} and Lubarsch^{37b} advanced the opinion that the multiple fatty growths in their cases were metastatic. Siegmund,^{37c} on the contrary, although labeling his case as one of "lipoblastic sarcoma," favored the conception of a "systemic disease of the fat tissue, the tumoral nature of which is unclear." The opinion of Siegmund was accepted by Goormaghtigh,²¹ who interpreted his own case similarly.

Both clinical course and morphologic aspects lead to a similar explanation in the case presented here. The process lasted twelve years after the appearance of the first fatty growth. A rather protracted clinical course is shown also in the other 5 cases of the literature in which the duration of the disease process is known, forty years in the case of Broca,^{37d} at least two or three years in the cases of Nienhuis^{37a} and of Siegmund^{37c} and a long unspecified period in the cases of Goormaghtigh²¹ and Askanazy,^{37e} the cause of death being attributed by both to an intercurrent infectious process.

In the present case, as in the cases of the others, the gross appearance of the fatty growths was also against the conception of them as cancer, there was, in fact, absence of nodular distribution, generally considered a characteristic of a neoplastic metastasis, and lack of evidence of vascular invasion by the proliferated fat tissue.

38 Stookey, B. *Arch Neurol & Psychiat* **18** 16, 1926.

39 Elsberg, C. A. *Surg, Gynec & Obst* **46** 1, 1928.



(See legend on opposite page)

The histologic observations further point against their cancerous nature. In the present case no evidence of cellular anarchy and no mitotic figures could be recognized in the numerous sections of the various fatty growths, which appeared to be mainly composed of adult fat cells, of embryonal fat cells and of less numerous undifferentiated mesenchymal cells, with all intermediate stages. Microscopic patterns of a similar nature are found in the cases of the other authors. In that of Askanazy^{37e} and that of Nienhuis^{37a} the fatty growths were likewise composed of mature fat cells and of undifferentiated mesenchymal cells, either sparse or in groups, with transitional forms between the two types. In Lübarsch's case^{37b} the structure was definitely that of mature fat cells, except for the nodules found within the bone marrow, which appeared unusually rich in young mesenchymal cells. In Siegmund's^{37c} and in Goormaghtigh's²¹ cases the structure fundamentally was one of irregularly disseminated embryonal mesenchymal cells, which were most numerous around branching blood vessels, with transitional forms from these cells to ripe fat cells.

Additional interest in the present case is offered by the foci of extra-medullary hemopoiesis present in most of the growths. This finding raises the question of the role of the embryonal mesenchymal cell of the fat tissue as a potential blood-forming cell (Wasserman,⁴⁰ Hubschmann⁴¹ and Gruber⁴²). The relationship between blood-forming bone marrow and adipose tissue is well known. That hematic cells are formed within fat tissue in various regions of the body seems to be the rule in

40 Wasserman, J. A. *Ztschr. f. Zellforsch. u. mikr. Anat.* **3**, 235, 1926.

41 Hubschmann. *Verhandl. d. deutsch. path. Gesellsch.* **19**, 236, 1923.

42 Gruber, C. B. *Ztschr. f. Kinderh.* **30**, 336, 1921.

EXPLANATION OF FIGURE

Left (a) Mature fat cells supported by a thin interlacing of connective tissue fibers, embedded in which fibroblasts, lymphocytes and younger mesenchymal cells with cytologic characteristics of undifferentiated mesenchymal cells are seen. From the primary popliteal mass removed at operation. (b) Mature fat cells showing great variations in size, irregularly intermixed with non-fat-bearing cells, among which young mesenchymal cells prevail over less numerous fibroblasts and lymphocytes. From a fat tissue new growth found in the mesentery at autopsy. (c) Mature fat cells being formed from periadventitial mesenchymal cells through increased storage of fat droplets, cellular swelling, withdrawing of cytoplasmic processes and, finally, peripheral displacement and flattening of the nuclei. From a fat tissue new growth, observed in the thoracic cavity at autopsy.

Right (a) Early stages of the process by which mesenchymal cells differentiate into fat cells. From a vascular area of the subcutaneous tissue of a human embryo 18 inches (45 cm.) long. (b) Progressive stages of fat cell maturation, from the undifferentiated mesenchymal cell containing a few cytoplasmic fat droplets to the ripe fat cell with nucleus displaced to the cell periphery. From the region of the thymus in a 2 day old baby. (c) Fat cells in acinous-like arrangement, embedded in the meshes of a thick capillary network. From the perirenal region of an 11 month old baby.

The figure is drawn from sudan III-stained preparations, objective 5, oil immersion Zeiss microscope.

embryonal life This type of hemopoiesis has been shown in the adult also, in pathologic states, the outstanding condition being leukemia Petri⁴³ has observed small patches of hemopoietic tissue in the retroperitoneal fat in a series of 40 persons, the majority dying of acute infections Foci of immature blood cells within a fat tissue tumor have been described by Blaisdell,⁴⁴ and Babes⁴⁵ mentioned normoblasts, megakaryocytes and plasma cells present in a recurrent mesenteric lipoblastoma In describing a highly cancerous nonlipid retroperitoneal tumor, Warren⁴⁶ stressed the presence of foci of immature hematic cells which he interpreted as originating from embryonal mesenchymal cells of the retroperitoneal fat tissue

The blood cell-forming potentiality of the undifferentiated connective tissue cells of the fat tissue, as shown in embryonal life and in pathologic states in the adult, might also explain the presence of immature hematic cells in the fat tissue growths in the case under consideration

HISTOGENESIS OF SYSTEMIC MULTICENTRIC LIPOBLASTOSIS AND COMPARISON OF THE DEVELOPMENT OF FAT TISSUE IN EMBRYONAL AND IN ADULT LIFE

Kolliker⁴⁷ was the first to advance the opinion that the fat cells originate from undifferentiated mesenchymal cells in which fat gradually is deposited The view of Kolliker has been accepted favorably by a majority, however, discrepancies arose in the identification of the lipogenetic connective tissue cell type According to Flemming,⁴⁸ fat imbibition is a potential function common to all connective tissue cells Mallory,⁴⁹ on the contrary, allocated this property to a specific cell set apart in early life for the sole purpose of storing fat Further studies by Hammar,⁴⁹ Chiari⁹ and Inglis⁵⁰ show that in regard to embryogenesis there are two sorts of fat tissue cells, both derived from the primitive mesenchyma In one type the differentiation becomes so well established that an irreversible and specialized tissue located in definite regions of the body is formed—mulberry fat tissue In the other type the fat tissue cells originate from ordinary connective tissue cells, which temporarily and as an adventitious function have taken over a certain amount of fat, to revert to their previous condition on loss of

43 Petri, E Virchows Arch f path Anat **258** 37, 1925

44 Blaisdell, J L Arch Path **16** 643, 1933

45 Babes, A Bull Assoc franç p l'étude du cancer **18** 334, 1929

46 Warren, S Am J Path **4** 51, 1928

47 Kolliker, A Anat Anz **1** 206, 1886

48 Flemming, W Virchows Arch f path Anat **52** 568, 1871

49 Hammar, J A Arch f mikr Anat **45** 512, 1895

50 Inglis, K J Anat **61** 452, 1927

the intracellular fat content Maximow⁵¹ and Wasserman⁴⁰ have more recently cast doubt on this conception. Maximow stated the belief that the specialized fat cell is entirely distinct from the fibroblast of the connective tissue, and as a proof of the high degree of differentiation of the fat cell he stressed its inability to multiply—hence his conclusion that both in embryonal life and in adult life fat tissue cells are formed from undifferentiated mesenchymal cells situated about the blood vessels. The conception of Wasserman is basically the same. He emphasized the interrelationship between the primitive fat organ, the small blood vessels and the “perivascular mesenchymal cells related to the reticulum,” to conclude that the adipose tissue is a differentiated part of the reticulo-endothelial system. In favor of this conception he mentioned the intimate relationship between hemopoietic tissue and fat tissue in the bone marrow, the formation of hematic cells in adipose tissue and the tendency of the lymphoid tissue to replace fat tissue and conversely (Askanazy^{37e} and Bufalini⁵²). The presence of argentaffin fibers, identified as pericellular reticulum (Volterra⁵³), and the property possessed by the embryonal fat tissue cell and, to a less extent, even by the mature fat cell to store vital dyes (Bremer,⁵⁴ Dogliotti⁵⁵ and Volterra⁵⁶) lend further support to Wasserman’s conclusion⁴⁰. Both Maximow⁵¹ and Wasserman⁴⁰ agreed on the point that fat storage in the course of cellular differentiation is accomplished through either local or general metabolic stimuli, the nature of which involves many unsolved problems. Under the influence of these stimuli the undifferentiated mesenchymal cells accumulate fat globules which, as maturation progresses, fuse together to give rise to the swollen spherical cell of the mature fat tissue. Withdrawing of the cytoplasmic processes and flattening and displacing of the nucleus against the cell membrane complete the cellular transformation.

This sequence of events was clearly shown by specimens of adult and embryonic fat tissue which were studied for the purpose of gaining personal acquaintance with the normal development of fat tissue. These observations are summarized briefly so that they may be compared with the findings in the fat tissue growths in the case of systemic multicentric lipoblastosis.

Human embryo 18 cm long (subcutaneous tissue). Within the primitive connective tissue, irregularly shaped areas were encountered which consisted of young mesenchymal cells and blood capillaries. Evidence of cytoplasmic

51 Maximow, B. *Textbook of Histology*, Philadelphia, W. B. Saunders Company, 1934.

52 Bufalini, M. *Arch ital di chir* **23** 281, 1929.

53 Volterra, M. *Sperimentale* **81** 319, 1927.

54 Bremer, J. L. *Anat Rec* **70** 263, 1938.

55 Dogliotti, G. C. *Ztschr f Zellforsch u mikr Anat* **8** 222, 1928.

56 Volterra, M. *Sperimentale* **77** 242, 1923.

storage of fat globules was noticed in a good number of these cells, suggesting early stages of the process by which mesenchymal cells differentiate into fat cells

Human embryo 29 cm long (perirenal region) In the meshes of a loose network of connective tissue fibers foci were encountered which were composed of young mesenchymal cells and blood capillaries The majority of these cells displayed cytoplasmic processes and large nuclei provided with nucleoli Within the cytoplasm of some of these cells minute fat droplets were seen, and where the fat droplets were larger and more numerous the cells appeared polygonal or rounded in shape

Mouse embryo 26 mm long (region of the thymus) All transitional stages were seen, from fully developed fat lobules, composed of large cells displaying cytoplasmic fat droplets and nucleus either central or displaced to the cell periphery, to small formations, in glandlike arrangement, consisting of cells in more or less advanced stages of fat maturation centered around the walls of branching blood capillaries These maturing fat cells were irregularly shaped, with large, centrally placed nuclei and various amounts of cytoplasmic fat droplets Undifferentiated mesenchymal cells with darkly stained nuclei and basophilic cytoplasm were also present

Baby 2 days old (periadrenal fat tissue) In between mature fat cells, numerous undifferentiated mesenchymal cells were seen, either sparse or in small groups, most numerous around the walls of blood capillaries Cytoplasmic fat droplets were recognized in some of these cells

Same baby (region of thymus) In the septums dividing the thymic lobules a fairly complete sequence of progressive stages of fat cell maturation was seen, from undifferentiated mesenchymal cells containing a few cytoplasmic fat droplets to ripe fat cells with nuclei flattened at the periphery

Baby 11 months old (periadrenal fat tissue) Large polygonal cells with central nuclei and small cytoplasmic fat-globules alternated with mature fat cells showing fusion of fat droplets and nuclei compressed at the periphery In between these mature or maturing fat cells, undifferentiated mesenchymal cells with dark-stained nuclei and basophilic cytoplasm were seen

Child 1½ years old (periadrenal fat tissue) In the center of a well developed, mature fat lobule there was a group of polygonal cells with large nuclei, two to three nucleoli and abundant cytoplasm Within the cytoplasm of these cells small fat globules were recognized

Rachitic child, age unknown (periadrenal fat tissue) In some areas mature fat cells, with large fat globules and nuclei flattened at the periphery, prevailed In some other areas polygonal cells with centrally placed, deeply stained nuclei were predominant Within the latter cells fat droplets of all sizes were seen, and by the confluence of these droplets the ordinary type of fat cell was seen to develop, the nucleus, which was centrally located as long as the individual droplets were discrete, appeared flattened at the periphery on complete maturation of the cell

White man 42 years old (pericardial fat tissue) The cause of death was phlegmon of the thigh Within the meshes of a cellular syncytium resulting from the fusion of the cytoplasmic processes of large stellate cells, abundant blood capillaries were encountered These were surrounded by thin mantles of young mesenchymal cells, which showed distinct cytoplasmic fat droplets In between these cells mature fat cells were seen There were concomitant myelocytes, normoblasts and other cell types suggesting extramedullary hematopoiesis

When the microscopic features found in maturing fat tissue both in embryonal and in adult life under normal conditions are compared with those of the fat tissue overgrowths of the case under study, the fundamentally identical plan of development is apparent, giving further evidence that the nature of the fat tissue overgrowths is more along the line of a hyperplastic process than of a neoplastic one in the strict meaning of the term

THE RELATION OF SYSTEMIC MULTICENTRIC LIPOBLASTOSIS TO LIPID STORAGE DISEASES

In recent years much interest has been aroused by a group of disease processes in which large amounts of lipid substances are accumulated in the histiocytes and in the cells of the reticuloendothelial system. It is not clear yet whether this is due to a primary disturbance in the metabolism of lipids which is expressed anatomically by such accumulation of the excessive lipid material carried in the blood stream or whether it is due to a dysfunction of the reticuloendothelial cells leading simultaneously to increased synthesis of lipids and to storing of these within the cells (Pick⁵⁷ and Thannhauser⁵⁸)

Further investigations have shown that clinical symptoms and pathologic findings are markedly different from case to case, according to the chemical composition of the accumulated lipids. Although overlapping of pathologic conditions and combinations of forms are not rarely encountered (Sobotka, Epstein and Lichtenstein,⁵⁹ Hertzog and colleagues⁶⁰ and Epstein⁶¹), studies along this line have led to the recognition of three main clinical pathologic entities: the cerebroside histiocytosis (Gaucher's disease), the phosphatide histiocytosis (Niemann-Pick disease) and the cholesterol histiocytosis, including Schuller-Christian disease and essential xanthomatosis.

In some of these disease processes—for instance, in Gaucher's disease—reticuloendothelial cells and histiocytes are selectively affected in determinate areas of the body. In other conditions, however—in essential xanthomatosis, for instance—the disease process manifests itself in a diffuse and unpredictable manner, bones, serosal membranes and internal organs being equally affected.

⁵⁷ Pick, L. *Am J M Sc* **185** 453, 1933

⁵⁸ Thannhauser, S. J. *Lipoidoses. Diseases of the Cellular Lipid Metabolism*, edited by H. A. Christian, New York, Oxford University Press, 1940

⁵⁹ Sobotka, H., Epstein, E. Z., and Lichtenstein, L. *Arch Path* **10** 677, 1933

⁶⁰ Hertzog, A. J., Anderson, F. G., and Beebe, G. W. *Arch Path* **29** 120, 1940

⁶¹ Epstein, E. *Virchows Arch f path Anat* **298** 430, 1937

Histochemical similarities have already suggested an intimate relationship between xanthoma and lipoma (Virchow⁶²) Hallopeau⁶³ and Torok⁶⁴ have considered the xanthomatous cells as embryonal fat cells, and Waldeyer⁶⁵ has shown that the first change in xanthoma consists in the appearance of fatty globules in perivascular mesenchymal cells, the same as that which occurs in the lipoblastic growths. Association in the same person of lipoma and xanthoma (Ehrmann⁶⁶) also points to an intimate relationship between the two processes.

Chemical investigations by Bonnefous and Valdiguié⁶⁷ showed high blood cholesterol in cases of diffuse lipomatosis with entirely subcutaneous distribution. Traina Rao,⁶⁸ in 10 cases of lipoma, found excessive lipids in the blood, including cholesterol, neutral fats, soap and phosphatides. Goormaghtigh and associates²¹ demonstrated that the mature fat cells of lipoma contain lecithin and cholesterol while the embryonal fat cells of lipoma contain neutral fat and fatty acids.

Since biochemical investigations were not made in our case or in the 6 similar cases in the literature, one can only wonder whether diffuse multicentric lipoblastosis also is due to altered metabolism of the lipid system, with incidental stimulation of undifferentiated mesenchymal cells leading to lipid storage, the whole resulting in tumor-like growths in multicentric areas.

SUMMARY

Multiple, recurrent nonencapsulated fat tissue growth, involving in a scattered and disorderly fashion both the subcutaneous tissue and the internal cavities of the body, was the outstanding characteristic of the case reported here. The process started in the popliteal space as an apparently simple lipoma, which was removed, the multicentric proliferation of fat tissue began six years later, and in spite of the numerous attempts to control the process by surgical intervention, a series of recurrences brought the patient to death in the course of twelve years.

Only 6 cases which might be compared with the one now reported were found in the literature, the majority under inconspicuous or misleading labels, such as "multiple lipoma," "lipoblastic sarcoma with metastasis," "metastasizing lipoma" or "lipoblastic sarcoma."

Distinctive characteristics emerging from the study of all these cases seem to point to a separate entity in the broad class of the lipid system overgrowths. The label "systemic multicentric lipoblastosis," proposed by Goormaghtigh,²¹ might be appropriate for this.

62 Virchow, R. *Virchows Arch f path Anat* **52** 504, 1871

63 Hallopeau. *Ann de dermat et syph* **4** 595, 1903

64 Torok. *Ann de dermat et syph* **4** 1109 and 1261, 1893

65 Waldeyer, W. *Virchows Arch f path Anat* **52** 318, 1871

66 Ehrmann, cited by Ewing²⁰

67 Bonnefous, R., and Valdiguié, A. *Ann de dermat et syph* **5** 290, 1924

68 Traina Rao, G. *Riv ital di ginec* **19** 1, 1936

Among the clinical-pathologic features common to these cases, the following are the most consistent (1) long duration of the disease process, (2) independent nonencapsulated fat tissue growths involving in an unpredictable and disorderly fashion subcutaneous tissue, internal cavities, bones and deep organs, (3) striking tendency of the fat tissue growths to recur after excision, (4) predominance in tissue of mature fat cells, with frequent transitional forms indicating that they originate from undifferentiated mesenchymal cells according to a plan which does not differ fundamentally from that by which fat tissue develops in either embryonal or adult life under normal conditions, and (5) lack of evidence of cellular anarchy and absence of mitotic figures and of any other cellular or structural pattern suggesting a neoplastic growth.

These being the main characteristics of the process, it seems unlikely that these cases belong in the category of cases of fat tissue tumors in the strict meaning of the term. Both for the case presented here and for the similar ones in the literature the possibility is conceived that the basic manifestation of the process is an alteration of the metabolism of the lipid system, with incidental stimulation of undifferentiated mesenchymal cells leading to lipid storage, the whole resulting in tumor-like growths in multicentric areas.

Case Reports

PRIMARY HODGKIN'S SARCOMA OF THE BRAIN

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SECONDARY lymphomatous involvement of the nervous system, though relatively infrequent, is well recognized. This subject has been adequately presented and thoroughly reviewed by Verda,¹ Browder and de Veer,² Ginsberg,³ Blakeslee,⁴ Von Hagen,⁵ Winkelman and Moore,⁶ Gray, Baker, Cottrell and Skogland⁷ and Jackson and Parker.⁸ In most of the cases reported by these authors the tumor metastasized to the brain by way of the blood stream, or, having invaded a skull bone, a vertebra or the spinal epidural space, involved the nervous tissue by direct extension or by compression. There are several cases in which the initial symptoms were referable to the nervous system, and the only clinically demonstrable tumor was in the spinal epidural space, the neoplasm having arisen there or having extended there from the vertebrae or from paravertebral tissues through the intervertebral foramina.

The possibility of a lymphoma arising in the substance of the brain and spinal cord has been hypothesized, but only a few examples of this have been recorded. Yuile⁹ and Kinney and Adams¹⁰ have reported 3 cases of primary reticulum cell sarcoma of the brain. The latter authors collected from the medical literature 5 similar cases which had been reported under the names "perithelial sarcoma," "microglioma" and "microglioblastoma." To date there have been no reported cases of Hodgkin's sarcoma, granuloma, or paraganuloma or of giant follicle lymphoma originating within the nervous system. Two cases of primary lymphosarcoma of the brain have been described by Abbott and

From the Mallory Institute of Pathology and the Neurological Unit, Boston City Hospital, and the Department of Neurology, Harvard Medical School.

1 Verda, D J. *M Clin North America* **24** 1228, 1944

2 Browder, J, and de Veer, J A. *Arch Neurol & Psychiat* **41** 328, 1939

3 Ginsberg, S. *Arch Int Med* **39** 571, 1927

4 Blakeslee, G A. *Arch Neurol & Psychiat* **20** 130, 1928

5 Von Hagen, K O. *Bull Los Angeles Neurol Soc* **20** 20, 1937

6 Winkelman, N W, and Moore, M T. *Arch Neurol & Psychiat* **45** 304, 1941

7 Gray, R C, Baker, A B, Cottrell, L, and Skogland, J E. *Clinics* **4** 230, 1941

8 Jackson, H, Jr, and Parker, F, Jr. *New England J Med* **233** 369, 1945

9 Yuile, C L. *Arch Path* **26** 1036, 1938

10 Kinney, T D, and Adams, R D. *Arch Neurol & Psychiat* **50** 552, 1943

Adson¹¹ In these cases the tumor appeared to have originated in the cerebral meninges, but it was impossible to exclude a skull bone as the primary source. With the permission of Dr J W Keenohan, we have examined microscopic sections in these 2 cases. The second case was unquestionably one of lymphosarcoma, the tumor in the first case, in our opinion, conforms in all respects to reticulum cell sarcoma. Unfortunately, a postmortem examination was not made in the case of lymphosarcoma. Therefore we must conclude that primary lymphosarcoma of the brain is not as yet an established pathologic entity.

In the past year at the Mallory Institute of Pathology a complete autopsy has been done in a case of primary Hodgkin's sarcoma of the brain. The patient had been admitted to the neurologic wards of the Boston City Hospital with symptoms of a tumor of the left frontal lobe. We are prompted to report this case because no report of one like it has appeared in medical literature.

REPORT OF A CASE

Approximately three months prior to entering the hospital the patient, a 53 year old white man, began to be drowsy and confused. These symptoms, at first intermittent, became more pronounced and persistent and were succeeded by a disturbance of speech, namely, slowness and difficulty in finding the correct words. Six weeks later he collapsed while at work. At this time he had a severe headache and an unsteady gait. Weakness of the right arm and leg developed soon afterward. Several times at night his wife noted jerking movements of his arms and legs, which were probably convulsions. Increasing confusion and nausea and vomiting led to his entering the hospital.

At the time of his admission the temperature, the pulse rate, the respiratory rate and the blood pressure were all within normal limits. The patient was drowsy and unable to give a satisfactory history. He was inattentive and forgetful and responded slowly and inadequately to all stimuli. There was spastic right hemiparesis affecting the face, the arm and the leg. The tendon reflexes of the right side were more active than those of the left, the right abdominal reflexes were absent, the right plantar reflex was extensor, whereas the left was flexor. The visual field and the optic fundi were normal, and there was no impairment of cutaneous sensation. The liver was enlarged, the lower edge being 7 to 8 cm below the costal margin.

The hemoglobin concentration was 80 to 90 per cent of normal. The white blood cell count and the differential count were within the limits of normal. A determination of the blood sugar showed 180 mg per hundred cubic centimeters during fasting. The Hinton test of the blood revealed no syphilis. The cerebrospinal fluid was under normal pressure and contained 10 lymphocytes per cubic millimeter and 333 mg of protein per hundred cubic centimeters, the colloidal gold curve was 5555443321, and the Wassermann and Davies-Hinton tests of the fluid were negative. No abnormalities were seen in roentgenograms of the skull, the spine, the chest, the pelvis and the proximal portions of the long bones. In an electroencephalogram taken on the ninth hospital day there were six to eight waves per second in all leads and marked asymmetry between the two

11 Abbott, K H, and Adson, A W. *Arch Surg* 47 147, 1943.

hemispheres, especially between the right and the left frontal lobe. The lower voltage and the less regular activity were over the left frontal lobe.

During the first eight days in the hospital the patient's condition became much worse. He was more confused and was unable to find correct words in attempts to express himself or to comprehend spoken words clearly. The right arm and leg became weaker and more spastic, and there was impairment of pain and of touch sensation over the right side of the body. He was incontinent of urine and feces.

On the tenth hospital day a ventriculogram was made, which disclosed that the lateral and third ventricles were slightly displaced to the right and that the anterior horn of the left lateral ventricle was obliterated. After this procedure the patient became comatose, and two days later craniotomy was done. The dura was found to be tense, and when it was reflected, there was exposed a firm, grayish white nodule in the superior frontal convolution, approximately 2 cm from the frontal pole. The anterior portion of the left frontal lobe was resected. After operation the patient remained in coma. Gradually the body temperature rose, the blood pressure fell and the breathing became stertorous, the patient died on the fifth postoperative day.

Surgical Specimen—The excised tissue showed a firm, grayish white mass, measuring 2 by 1.5 cm, embedded in edematous brain tissue. On the cut surface the mass was well demarcated from the surrounding brain tissue and was flecked with minute red and yellow areas.

Tissue from the tumor was fixed in Zenker's fluid and in solution of formaldehyde U S P diluted 1 to 10 and was stained with phloxine-methylene blue, Foot's modification of Hortega's stain for reticulum and Mallory's phosphotungstic acid-hematoxylin and aniline blue stains for collagen.

In microscopic sections large masses of tumor cells replaced the cerebral cortex and white matter. Under low magnification the tumor presented many well preserved areas separated by broad bands of necrotic tissue. The viable tumor consisted of sheets of cells approximately 15 microns in diameter, without syncytial relations and forming loosely arranged groups and clusters. Viable cells tended to be grouped around blood vessels. The nucleus of the tumor cell was large and round to ovoid and contained moderately coarse, evenly dispersed particles of chromatin, from one to three prominent nucleoli were visible, the cytoplasm was relatively sparse and palely basophilic. Single lobulated, binucleated and multinucleated giant cells were abundant (fig 1). Numerous mitotic figures were present. No glial fibrils were seen. There was an abundance of reticulum in the form of a network of fibers which surrounded single cells and small clusters of cells (fig 2). Sparsely scattered collagen fibers were found throughout the tumor.

Autopsy (two and one-half hours after death)—When the incision of the scalp was opened, a small amount of malodorous grayish purulent material was exposed. This exudate had collected on the external surface of the dura and to a slight extent in the subdural space. The leptomeninges were opaque, and purulent exudate was seen in the subarachnoid space. The brain weighed 1,350 Gm. There was a large defect in the left frontal lobe. The adjacent brain tissue was soft, granular and hemorrhagic. No tumor tissue could be identified. The choroid plexuses were covered with purulent exudate, and there were numerous petechial hemorrhages beneath the ependyma of the posterior horns of the lateral ventricles. The dural sinuses, the bones of the base of the skull and the paranasal sinuses revealed no evidence of tumor.

There was fibrous pleuritis on the right side. The liver weighed 2,200 Gm. Its surface was pale and presented nodules varying up to 2 mm in diameter. It sectioned with increased resistance, and on the cut surface there were similar yellow nodules separated by very thin gray fibrous bands. There were no

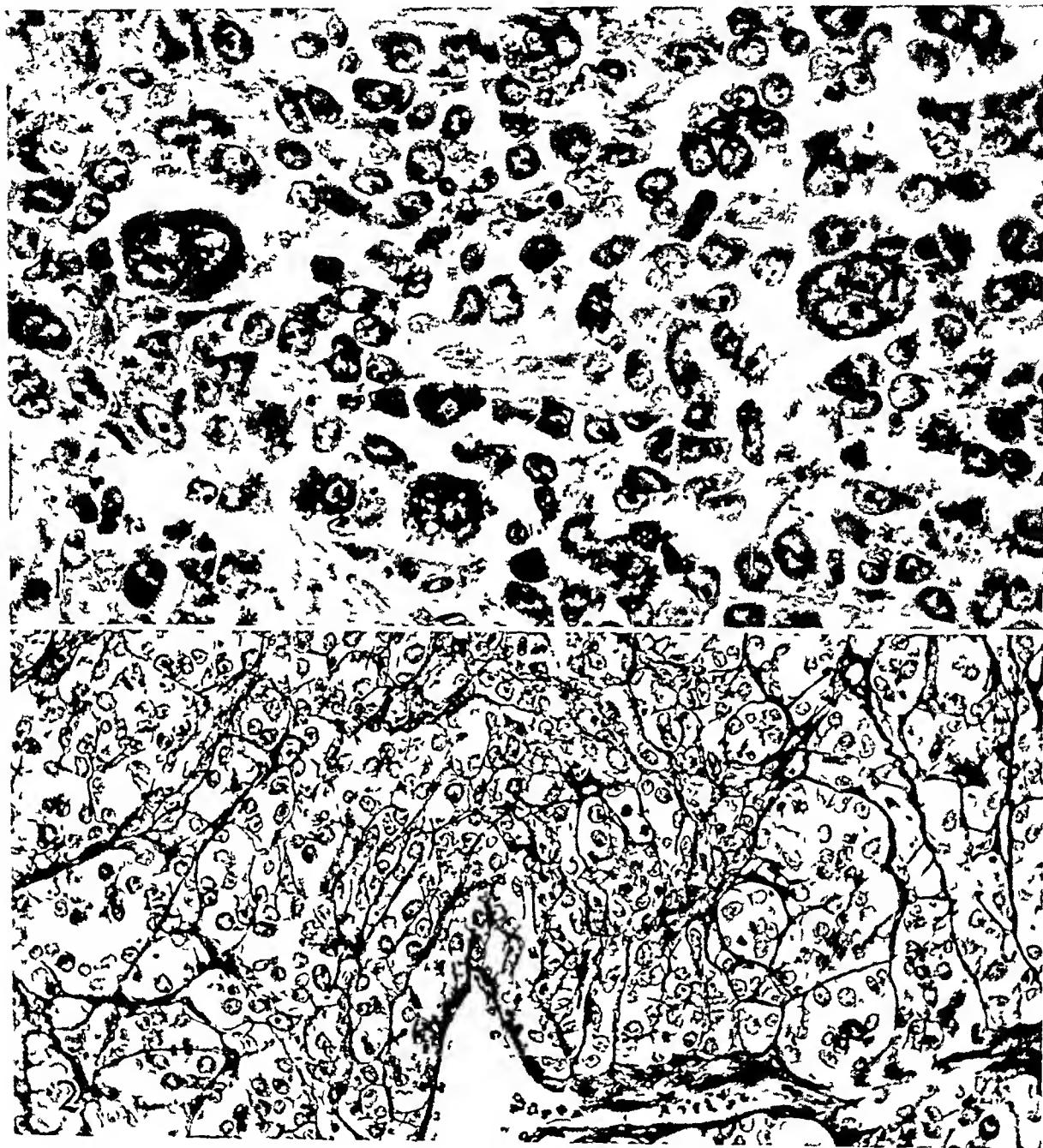


Fig 1—Section of tumor showing characteristics of sarcomatous growth, tumor cells with large nuclei and prominent nucleoli, and three multinucleated Reed-Sternberg giant cells. Phloxine-methylene blue, $\times 780$.

Fig 2—Section of tumor showing reticulum fibers distributed between groups of cells and individual cells. Foot's modification of the Hortega stain for reticulum, $\times 280$.

enlarged lymph nodes Several small abscesses were found within the substance of the prostate gland Postmortem roentgenograms of the bones of the extremities revealed no abnormalities

Bacteriologic Report—*Escherichia coli* and hemolytic *Staphylococcus aureus* were isolated from the exudate over the dura, and *E coli* and *Bacillus proteus* from the subarachnoid space A few colonies of *Staph aureus* were obtained on culture of the heart's blood In cultures of the lung there were a few colonies of *B proteus* and hemolytic *Staph aureus* Similar organisms were obtained from the prostate gland

Microscopic Examination—Only the brain and the liver were of significant interest The exudate on the outer surface of the dura contained fibrin, many polymorphonuclear leukocytes and bacteria, both rods and cocci A similar exudate was attached to the inner surface of the dura The cerebral and spinal leptomeninges were infiltrated by large numbers of polymorphonuclear leukocytes and a few lymphocytes, and a similar exudate had collected in the subarachnoid space No tumor cells were present in either the brain or the meninges In sections of the brain tissue adjacent to the excised area there was widespread necrosis of nerve and glial cells, with many petechial hemorrhages, as well as an infiltrate of polymorphonuclear leukocytes

The normal structure of the liver was distorted by thin fibrous septums, the presence of which resulted in the formation of small nodules The increased fibrous tissue was primarily portal in distribution Many hepatic cells contained large or small vacuoles Alcoholic hyalin was not identified

The remaining histologic sections served only to confirm the other gross findings, which were of minor importance

Anatomic Diagnosis—(1) Primary Hodgkin's sarcoma of the left frontal lobe of the brain, (2) acute purulent pachymeningitis, leptomeningitis and localized bacterial encephalitis, postoperative, (3) alcoholic cirrhosis of the liver, (4) pulmonary atelectasis, emphysema and congestion, and (5) acute prostatitis with abscess formation

COMMENT

The principal symptoms in this case were confusion, partial motor aphasia and right hemiparesis They clearly indicated extensive damage of the left frontal lobe In view of the age of the patient and the fairly rapid and progressive evolution of the symptoms it was obvious that there was a rapidly growing tumor of the brain even though the cerebrospinal fluid pressure was normal There was no sign of carcinoma of the lungs or other viscera, hence the clinical diagnosis was glioblastoma multiforme The terminal elevation of temperature and the persistent coma were probably related to the infection of the wound and the acute bacterial leptomeningitis

The tumor tissue had the usual gross appearance of Hodgkin's sarcoma It was firm, homogeneous and in places flecked with red and yellow areas Histologically the tumor was characteristic of Hodgkin's sarcoma The cell type was a large mononuclear cell with a large nucleus and prominent nucleoli Reed-Sternberg giant cells were abundant, and the reticulum formed a delicate intercellular stroma

That the brain was the site of origin seems indisputable. There was no clinical evidence of involvement of cervical lymph nodes, of paranasal sinuses or the nasopharynx, of other viscera or of bones. Finally, postmortem roentgenograms of the long bones and a complete autopsy failed to show tumor in other organs from which a cerebral metastasis could have arisen.

Although histologically separable, Hodgkin's sarcoma and reticulum cell sarcoma often have a similar clinical course. It is of interest in this respect to compare our case with the 2 cases of primary reticulum cell sarcoma of the brain reported by Kinney and Adams¹⁰ and the 5 cases which they collected from the literature. All the patients with reticulum cell sarcoma were males, varying in age from 9 years to 72 years, with an average age of 44 years. The average duration of symptoms prior to operation was approximately six months, and the period of survival after the operation averaged three months. In all these patients the tumor was situated in the temporal lobe. Similarly, our patient with Hodgkin's sarcoma was old, and the clinical course was brief (three months). In contrast to the reticulum cell sarcoma, which in all 7 cases was located in the temporal lobe, the tumor in our case was situated in the frontal lobe.

The precise cell type of Hodgkin's sarcoma is not definitely known. Some authorities believe that both reticulum cell sarcoma and Hodgkin's sarcoma are derived from the reticulum cell or the histiocyte and differ from each other only in minor details. In other words, these tumors are essentially two forms of a histiocytic sarcoma. If this assumption is correct, Hodgkin's sarcoma of the brain would be expected to arise from histiocytic cells of the meninges or the adventitia of the blood vessels. A third hypothetical source would be the microglial cells, which, according to the studies of Hortega,¹² are derived during fetal life from histiocytes at certain fixed points in the leptomeninges. It is not intended in the foregoing discussion to suggest that the pathologic distinction between reticulum cell sarcoma and Hodgkin's sarcoma should be abandoned. The occurrence of primary reticulum cell sarcoma of bone with its relatively good prognosis, contrasted with the absence of primary involvement of bone in Hodgkin's sarcoma, and the not infrequent transition of Hodgkin's granuloma to Hodgkin's sarcoma but never to reticulum cell sarcoma make a distinction between these two tumors important.

In recent years 2 cases of cerebellar involvement by a malignant tumor with the histologic characteristics of Hodgkin's sarcoma have been seen at the laboratory with which we are associated. One of

¹² del Rio Hortega, in Penfield, W. Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932.

these cases has been previously reported in detail⁸ In both, the short duration of the clinical course and the initial signs and symptoms of a rapidly growing intracranial malignant neoplasm were similar to the case reported here Careful clinical study detected no involvement of lymph nodes, nasopharynx, viscera or skeleton by a malignant tumor which might have given rise to a cerebral metastasis Necropsy showed a malignant tumor of the cerebellum with the characteristic microscopic appearance of Hodgkin's sarcoma Unfortunately, only the brain was examined in each instance Although the clinical evidence indicates that these 2 cases were examples of primary Hodgkin's sarcoma of the cerebellum, a definite conclusion cannot be drawn because of the lack of complete pathologic confirmation

SUMMARY

To the best of our knowledge the case of Hodgkin's sarcoma of the left frontal lobe of the brain which we have described is the first case of primary Hodgkin's sarcoma of the brain to be reported In addition, 2 cases of Hodgkin's sarcoma of the cerebellum have been encountered, but the proof of the site of origin is not conclusive since only the brain was submitted for pathologic examination

PRIMARY MELANOMA OF THE ADRENAL GLAND

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PRI-MARY melanoma of the adrenal gland is very rare. Only a few reports of melanoma that can be considered of true adrenal origin are to be found in the literature. As Goldzieher¹ pointed out, the diagnosis requires great caution because of the possibility that a small unrecognized primary tumor of the eye or the skin is present with predominant secondary involvement of the adrenal gland. The histogenesis of such a primary growth has interested many workers and will be discussed later in this paper.

LITERATURE

In 1914 Pappenheimer² reported a case of primary melanoma of the adrenal gland. At that time he accepted only 5 cases, which had included references to the eyes, as adequately reported. These were the cases of Schweikert, Davidsohn (case 2), Schumm and Molnar and his own case. Goldzieher considered as authentic the cases of Davidsohn, Schumm, Neuberg, Goldzieher, Pappenheimer, Schmidt and MacLachlan. The case reported by MacLachlan³ in 1915 was that of a bilateral melanotic growth with extensive metastasis. In 1927 R. M. Smith⁴ described a primary melanotic growth of the adrenal glands. In 1933 McComb and D. B. Smith⁵ reported a case of primary bilateral malignant tumor of the adrenal glands with secondary melanotic growths in the subcutaneous tissue and the urinary bladder. The primary neoplasm did not contain melanin. In 1938 Baker⁶ described a pigmented adenoma of the adrenal gland. The pigment reacted to various reagents as melanin does, but the tumor did not have the characteristic gross or microscopic structure of melanoma, nor was there evidence of tumor elsewhere. It is fairly certain that there are not more than 11 authentic cases reported. Recently we encountered a case of primary cancerous melanoma of the adrenal gland.

From the Section on Pathologic Anatomy, Mayo Clinic

1 Goldzieher, M. A. *The Adrenal Glands in Health and Disease*, Philadelphia, F. A. Davis Company, 1944, p. 104.

2 Pappenheimer, A. M. *Proc. New York Path. Soc.* **14**, 173, 1914.

3 MacLachlan, W. W. G. *J. M. Research* **33**, 93, 1915.

4 Smith, R. M. *M. J. Australia* **1**, 683, 1927.

5 McComb, R. A., and Smith, D. B. *J. Urol.* **30**, 49, 1933.

6 Baker, M. R. *Arch. Path.* **26**, 845, 1938.

REPORT OF A CASE

The patient was a 60 year old white man who registered at the Mayo Clinic first on July 12, 1944 with symptoms of duodenal ulcer which had been diagnosed and treated elsewhere for two years. He returned July 26, 1945, with intermittent pain over the lower part of the back which extended down the thighs. He complained of anorexia and had lost 40 pounds (18.1 Kg) during the previous year.

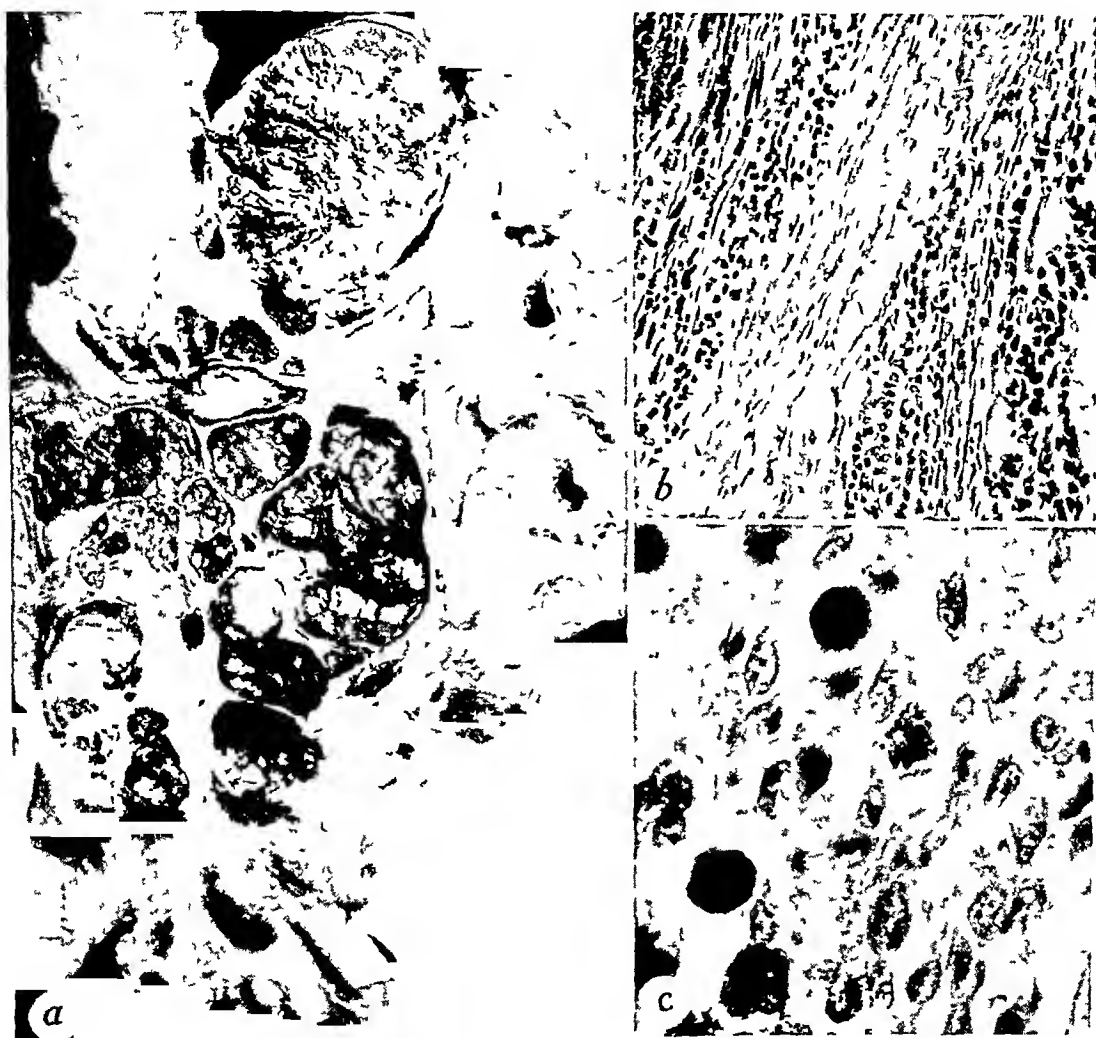


Fig 1—Melanoma of the left adrenal gland (a) Gross view of the growth with metastases in lymph nodes (b) Histologic structure of the primary neoplasm ($\times 145$) Compressed cortex is visible (c) Structure of the cancerous cells ($\times 475$) The melanin pigment is seen in a number of these cells

The essential finding at examination was a palpable, questionably retroperitoneal mass in the midabdomen. Tenderness over the fourth lumbar vertebra was present. The roentgenograms revealed destruction of the fourth lumbar vertebra. Roentgen therapy was given to the lumbar region, with relief of pain. On September 12, coma ensued with auricular fibrillation, cyanosis and a fall of blood pressure to 50 mm of mercury systolic and 40 diastolic. Death occurred on September 12.

Necropsy—The body was moderately emaciated. A tumor mass measuring 8.5 by 5.5 by 4.5 cm was present at the superior pole of the left kidney (fig 1*a*). It was brownish black and soft, though not friable. It appeared to have originated from and destroyed the left adrenal gland. Numerous pigmented tumor masses surrounded the superior, medial and inferior borders of the left kidney. Some of these masses showed central necrosis. Five centimeters above the point where the left ureter entered the bladder, pigmented tumor nodules obstructed the lumen. Above this obstruction, the ureter and the renal pelvis were moderately dilated. Infected hydronephrosis with abscess of the left kidney was present. The right adrenal gland contained a small pigmented tumor nodule and was moderately atrophied.

The skin showed no pigmented growths. The brain and the spinal cord and their meninges were normal. The choroid and the retina of each eye were removed, examined closely and found to be normal.

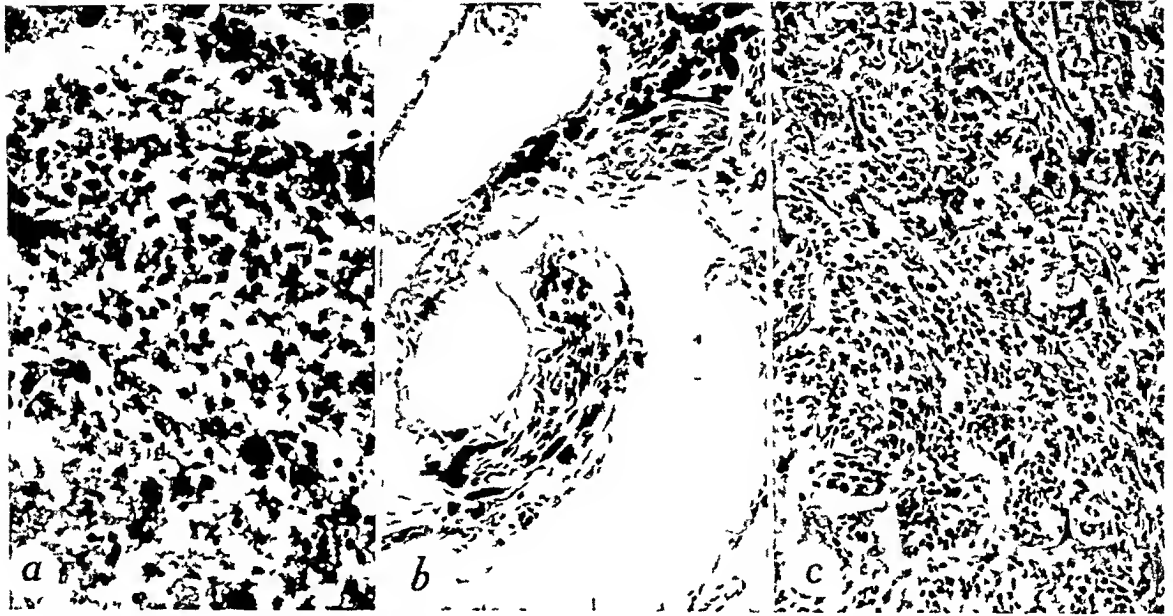


Fig 2—Metastatic lesions. (a) Metastatic neoplasm of the right adrenal gland treated with Becker's silver nitrate reagent. Note the reduction of silver nitrate ($\times 200$). (b) Pigmented metastasis in the lung ($\times 140$). (c) Metastatic neoplasm in the liver. The cells are similar to the neoplastic cells elsewhere, but no pigment is present ($\times 95$).

The superior mediastinal lymph nodes were enlarged and contained melanotic tumor masses. In the liver there were smooth, yellowish white, sharply circumscribed tumor nodules, measuring 1.0 to 3.0 cm in diameter. They were present both on the surface and in the substance of the liver. The intestinal tract showed no visible abnormalities, but lymph nodes in the region of the head of the pancreas were involved with pigmented metastatic growths. Pigmented metastatic growths were present in the bodies of the twelfth thoracic and the first, second, third, fourth and fifth lumbar vertebrae. Scarring of the duodenum 1 cm distal to the pylorus was observed and interpreted as evidence of a healed duodenal ulcer. Edema of the lungs and hydrothorax (250 cc on the left and 100 cc on the right) also were present.

Histologic Examination—The primary tumor was composed of sheets of spindle-shaped cells with many regions of necrosis (fig 1b). The tumor cell (fig 1c) had a prominent oval to spindle-shaped nucleus with a reticular network and pink granular cytoplasm with an indistinct border. Mitotic figures were numerous, and many of the nucleoli were large and prominent. Large numbers of cells contained coarse, golden brown pigment granules in the cytoplasm. In many areas the pigment was so abundant that cellular details could not be seen. The Berlin blue reaction for iron with a counter stain of basic fuchsin was negative, however, the pigment was blackened when Becker's silver nitrate solution was used as reagent. The dark color of melanin is brought out by oxidizing agents. Mucus was not present in sections treated with the Dresbach⁷ modification of Meyer's mucin stain. No production of reticulum was seen in tissue stained by the Gomori reticulum method. An associated acute inflammatory reaction appeared in the scattered necrotic areas.

The metastatic tumors in the regional nodes, the right adrenal gland, the peri-ureteral region and vertebrae which were noted grossly corresponded microscopically to the histologic picture described in the preceding paragraph (fig 2a). Metastatic lesions also were detected in the lungs (fig 2b) and the prostate on histologic examination of these organs. The nodules in the liver (fig 2c) were made up of cells which resembled the ones described, but no production of pigment was observed.

COMMENT

Because of the rarity of primary melanoma of the adrenal gland, a thorough search was made for a commoner primary source such as the eye or the skin. There was no primary neoplasm in the skin, nor was there a history of the removal of a mole. There were no ocular symptoms, and, as noted previously, the choroid coats were examined carefully and found to be normal. The rectum and the anus were free of tumors, as were the meninges and the central nervous system. Because of the absence of any other identifiable origin and because of the large size of the tumor in the left adrenal gland, with its local nodal involvement and distant metastatic lesions, we felt justified in considering this as a case of cancerous melanoma primary in the adrenal gland. The tumor was identified to our satisfaction as a melanin-producing neoplasm by the use of the Berlin blue reaction for iron and Becker's silver nitrate reagent. These were deemed to give adequate evidence of the nature of the pigment, since Baker has shown that only these two reagents give any valuable histologic information regarding melanin-like substances. Bloch's "dopa" was not available, and since the necropsy was performed after vascular embalming, the reaction with this reagent would have been of no aid in identification.

As has been true of other investigators of the subject, we have had our curiosity aroused regarding the histogenesis of tumors of this type. Conflicting views have been presented in the literature. Most of those who have worked on the problem agree that epinephrine is produced in the adrenal medulla and that a close chemical relation exists between epinephrine and melanin. Epinephrine has been oxidized to a melanin-like pigment by a variety of methods. Edlbacher⁸ cited the work of

⁷ Dresbach, M. Personal communication to the authors.

⁸ Edlbacher, S., in Luck, J. M. *Annual Review of Biochemistry*, Stanford University, Calif., Stanford University Press, 1937, vol. 6, pp. 275-277.

Schuler and his associates, who concluded that tyrosine is the mother substance of epinephrine. Tyrosine when oxidized by the enzyme tyrosinase yields melanin pigment. It is therefore natural to postulate that the cells of the adrenal medulla are the source of the melanoma, since these cells are the producers of epinephrine. Kaufmann⁹ referred to groups of melanotic pigmented ganglion cells in the medulla arising from the sympathetic formative cells. These could conceivably serve as the predecessors of the tumor. In support of this concept, Millar¹⁰ has reported a cancerous melanotic tumor of the ganglion cells, arising from a thoracic ganglion. McComb and Smith offered the pheochromocyte as the possible cell of origin of the neoplasm in their case of melanotic tumor. MacLachlan, however, was not convinced of this source, because of the appearance of the cells of the tumor that he reported. He has advocated the chromatophore, which is found in the loose connective tissue about the adrenal gland as the source. Goldzieher was impressed with the similarity of structure between the melanotic and the unpigmented cortical tumors, and expressed the belief that the cells of the cortex are the source of these tumors. He also pointed out the fact that benign adenoma of the cortex is by no means rare.

We have occasionally observed small, brownish black nodules, so-called melanomas, in the adrenal cortex and wondered whether one of these might possibly have been the source of the neoplasm described. An investigation of a number of these nodules in 21 other cases was carried out. The pigment was found to have some of the properties of a lipid, but no melanin could be identified. This led us to abandon the idea that these small pigmented cortical tumors might be the predecessors of cancerous melanoma. It would seem that the precise cell of origin in cases of cancerous melanoma of the adrenal gland is still in doubt.

SUMMARY

Various sources for the cell of origin of cancerous melanoma of the adrenal gland have been postulated but our observations in a case of primary cancerous melanoma of the left adrenal gland with widespread metastatic growths did not allow a conclusion as to the histogenesis of the tumor.

9 Kaufmann, E. *Pathology for Students and Practitioners*, translated by S. P. Reimann, Philadelphia, P. Blakiston's Son & Co., 1929, vol. 2.

10 Millar, W. G. *J. Path. & Bact.* **35**: 351, 1932.

Laboratory Methods and Technical Notes

A CENTRIFUGE METHOD OF DETERMINING BLOOD PROTEINS

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SEVERAL methods have been described for the estimation of the protein of urine and cerebrospinal fluid in which the volume of precipitated protein is measured after the urine or the fluid has been centrifuged in special graduated tubes (Shevky and Stafford¹, Young and Bennett², Young, Bennett, Christlieb and Myers³, McNaught⁴, Bauer and Schenck⁵). Experiments have been carried out which show that the principle of these methods can be used in the estimation of the total protein, the albumin and the globulin of blood serum. Shevky and Stafford suggested that their method be used for estimating the protein of serum and other body fluids but gave results of its use only with urine. No way of standardizing the procedure other than timing the period of centrifugation has been given. These investigators claim only approximately accurate results. A calibrated centrifuge tube was used by them, but they gave no description of it. Peters and Van Slyke⁶ pictured a graduated tube described in a personal communication from McKay, whose results for the protein of urine when he used this tube in a modification of the Shevky and Stafford method were accurate to within 10 per cent.

Of the special centrifuge tubes described, that of Bauer and Schenck appears to be the one best adapted for use in measuring the volume of precipitated serum protein. Its construction is such that there is small likelihood of the precipitate being impacted in the shoulder of the tube and thus prevented from entering the tip. The long, narrow, tapered tip holds 0.4 cc. and is graduated to 0.004 cc. Estimations of 0.002 cc. can be easily made. The accuracy of the graduations of tubes obtained at a local supply house has been checked and found satisfactory. A minor disadvantage is their total capacity of only 3 cc.

Comparative experiments have shown that the tungstic acid reagent of Folin and Wu is the most suitable precipitant. It produces a finely granular soft precipitate that packs evenly on being centrifuged. Its

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This investigation was aided by a grant from the Otho S. A. Sprague Memorial Institute for Medical Research.

1 Shevky, M. C., and Stafford, D. D. *Arch. Int. Med.* **32**: 222, 1923.

2 Young, G. A., and Bennett, A. E. *Am. J. M. Sc.* **172**: 249, 1926.

3 Young, G. A., Bennett, A. E., Christlieb, J. M., and Myers, J. T. *Arch. Neurol. & Psychiat.* **23**: 542, 1930.

4 McNaught, J. B. *J. Lab. & Clin. Med.* **16**: 999, 1931.

5 Bauer, A. R., and Schenck, H. P. *J. Lab. & Clin. Med.* **16**: 1090, 1931.

6 Peters, J. P., and Van Slyke, D. D. *Quantitative Clinical Chemistry*, Baltimore, Williams & Wilkins Company, 1932, vol. 2.

further advantage is that it is the precipitant usually employed in the chemical estimation of serum protein, this being the method by which the centrifuge method is standardized

PROCEDURE

The precipitant is prepared by mixing 1 part of 10 per cent sodium tungstate, 6 parts of distilled water and 1 part of two thirds-normal sulfuric acid. This mixture can be kept for two weeks, after which it must be discarded and a fresh one prepared (Van Slyke and Hawkins⁷). One and eight-tenths cubic centimeters of the solution is measured into the Bauer-Schenck tube. It is best that the precipitant be not allowed to enter the narrow tip of the tube, in order to facilitate future mixing. With a small bore accurate pipet 0.2 cc. of clear serum is now measured into the tube. The mixture is stirred well with a thin glass rod, the stirrer making certain that none of the precipitate adheres to the glass walls of the tube. One more cubic centimeter of the precipitant is added, which is used to wash the glass rod and bring the volume to 3 cc. A sharp tapping of the tube fills the tip and further mixes the contents. The tube is capped with a sheet of

TABLE 1—*Volumes of Protein Precipitated from Serum Treated by the Centrifuge Method*

| Samples of Serum with 7.1 per Cent Protein, Cc | Percentage of Protein If Sample Were Contained in 0.2 Cc | Volume of Precipitate, Cc |
|--|--|---------------------------------|
| 0.22 | 7.81 | 0.336 |
| 0.20 | 7.10 | 0.306 |
| 0.18 | 6.39 | 0.276 |
| 0.16 | 5.68 | 0.246 |
| 0.14 | 4.97 | 0.216 |
| 0.12 | 4.26 | 0.186 |
| 0.10 | 3.55 | 0.156 |
| 0.08 | 2.84 | 0.126 |

cellophane held in place by a rubber band. The tube is then centrifuged for fifteen minutes at full speed, the centrifuge being started as rapidly as can be safely done. With the aid of a magnifying glass, the volume of the precipitate is read.

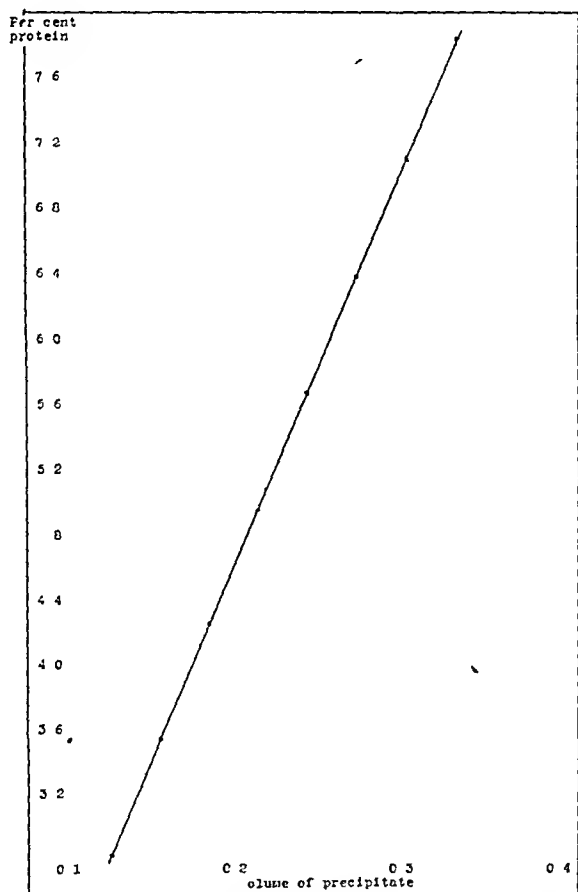
In order to find the quantitative relationship of the volumes of various amounts of protein precipitated from serum treated according to the procedure described in the foregoing paragraph, a serum containing a 7.1 per cent protein as determined by the Kjeldahl method was used in amounts of 0.22, 0.2, 0.18, 0.16, 0.14, 0.12, 0.10 and 0.08 cc. The volume of protein contained in these samples corresponds to that in 0.2 cc. amounts of serums whose protein contents are, respectively, 7.81, 7.10, 6.39, 5.68, 4.97, 4.26, 3.55 and 2.84 per cent. The volumes of protein obtained are given in table 1. When these volumes are plotted against the percentages of protein, a straight line results (chart).

It will be noted that the volume of precipitate is large relative to the volume of the sample used. This is due to the high hydration of the precipitated protein. By prolonged centrifugation the water content can be materially reduced. A series of precipitates similar to the ones obtained in this experiment were centrifuged for one hour and thirty minutes at full speed with interruptions at fifteen minute intervals for readings. These showed that the volume is reduced rapidly during the first fifteen minute periods and that although reduction was progressively slower during each succeeding period there was still a significant reduction during the

⁷ Van Slyke, D. D., and Hawkins, J. A. *J. Biol. Chem.* **79** 739, 1928

sixth and final period. However, the volumes of the precipitates at each reading when plotted against percentages of protein formed a straight line curve. These lines changed in slope during the first three periods but were parallel during the remaining periods.

Many experiments have shown that the centrifugal force developed by a centrifuge cannot be duplicated on successive runs even though the time of centrifugation is made as nearly the same as possible. Varying amounts of precipitated protein set up in duplicated lots and centrifuged for identical periods yielded results which were variable and which gave straight line curves with different slopes, none of



The volume of protein precipitated from a serum treated by the centrifuge method plotted against the percentages of protein shown in table 1

which attained zero when projected. Therefore, when the protein content of an unknown serum is to be determined, it is necessary to use in the same experiment at least two different amounts of a serum with known protein content in order to obtain a curve characteristic of that run of the centrifuge. To this end a standard serum whose protein content was carefully established by the Kjeldahl method was distributed in 0.5 cc amounts in small vials stoppered with paraffined corks and kept frozen. For each lot of determinations on unknown serums the content of one of these vials was thawed, and 0.2 and 0.1 cc samples were carried through the procedure along with 0.2 cc samples of the unknown serums. A straight line curve

was constructed from the results with the standard serum. Along this curve the volumes of precipitate from the unknown serums were located and the percentages of protein read.

For the determination of the globulin and the albumin of serum, the globulin is removed from the serum by half-saturation with ammonium sulfate, and the volume of the precipitate representing the remaining albumin is determined in the same manner as that of total protein. A sample of serum is well mixed with an equal amount of saturated solution of ammonium sulfate and centrifuged. The clear supernatant is pipeted off, and 0.4 cc samples are used for the estimation of albumin. This amount contains the albumin remaining in 0.2 cc of the serum after removal of the globulin in the manner described. From the volume of precipitate the percentage of protein as albumin is determined, the curve established with two amounts of standard serum being used as for estimation of total protein. It is necessary, of course, that this curve be constructed from readings made after the standard precipitates have been centrifuged along with the unknown albumin precipitate. Usually total protein and albumin are determined together. Sub-

TABLE 2—*Comparison of Results Obtained with the Centrifuge and the Kjeldahl Method*

| Centrifuge Method | | | Kjeldahl Method | | |
|-------------------|---------|----------|-----------------|---------|----------|
| Total Protein | Albumin | Globulin | Total Protein | Albumin | Globulin |
| 7.16 | 5.09 | 2.07 | 7.40 | 5.36 | 2.04 |
| 7.36 | 5.18 | 2.18 | 7.44 | 5.22 | 2.22 |
| 6.02 | 3.90 | 2.12 | 6.14 | 4.10 | 2.04 |
| 7.18 | 4.67 | 1.51 | 7.54 | 4.92 | 2.62 |
| 7.09 | 4.91 | 2.18 | 7.28 | 5.12 | 2.16 |
| 7.26 | 5.18 | 2.08 | 7.18 | 5.30 | 1.88 |
| 7.04 | 5.28 | 1.76 | 7.18 | 5.52 | 1.66 |
| 6.99 | 5.10 | 1.89 | 7.20 | 5.46 | 1.74 |
| 7.00 | 4.51 | 2.49 | 6.86 | 4.72 | 2.14 |
| 7.23 | 5.31 | 1.92 | 7.44 | 5.66 | 1.78 |
| 7.10 | 5.10 | 2.00 | 7.51 | 5.37 | 2.14 |
| 6.82 | 4.91 | 1.91 | 7.16 | 5.08 | 2.08 |
| 5.81 | 3.76 | 2.05 | 6.04 | 4.00 | 2.04 |
| 6.44 | 4.81 | 1.63 | 6.48 | 4.96 | 1.52 |
| 7.16 | 5.02 | 2.14 | 7.68 | 5.44 | 2.24 |

tracting the percentage of albumin from the percentage of total protein gives the percentage of globulin.

Removal of globulin with sodium sulfate according to the Howe method⁸ was considered, but the use of ammonium sulfate was believed to be preferable. When protein is determined by its content of nitrogen the use of ammonium sulfate is interdicted. With the centrifuge method the nitrogen of ammonium sulfate is of no concern. When sodium sulfate is employed as described by Howe to remove globulins, the serum is diluted too much for convenient use with the centrifuge method. Moreover, the necessity of a period of three hours at 37°C for precipitation of globulins is avoided when ammonium sulfate is used. Also the loss of albumin by adsorption on filtration, even when the best grade of filter paper is used,⁹ and the error introduced by evaporation on filtration in a warm chamber are eliminated when ammonium sulfate is substituted for sodium sulfate.

⁸ Howe, P. E. *J. Biol. Chem.* **49**, 93, 1921.

⁹ Robinson, H. W., Price, J. W., and Hogden, C. G. *J. Biol. Chem.* **120**, 481, 1937.

In table 2 are given the comparative results of a series of determinations of serum proteins made by the centrifuge method and by the Kjeldahl method, protein fractionation being made in the former with ammonium sulfate and in the latter with sodium sulfate as described by Howe. The greatest departure of the results for total protein with the centrifuge method from those with the Kjeldahl method was 5.8 per cent, the average being 3.2 per cent. For albumin the greatest departure was 7.0 per cent, with an average of 4.2 per cent.

The chief precautions to be taken in this method are: 1. Identical procedures must be used with each of the tubes in the same run of the centrifuge, including the speed and the manner of introducing the serum samples into the precipitant. 2. The measurements and the manipulations of each tube should be carried out as nearly together as possible. With observation of these two rules, duplicates are usually unnecessary. When total protein and albumin are determined on a single serum four places in the centrifuge are used. For the same determinations on each other serum run at the same time two more places are used. With a centrifuge having eight places total protein and albumin of three serums or total protein alone of six serums can be determined.

The following is a recapitulation of the method in outline form:

| | | | |
|------------------------------|--|---|---|
| Tube 1 | Measure into each 1.8 cc of precipitant | Add 0.2 cc standard serum | Mix contents of tubes thoroughly. Centrifuge 15 minutes at full speed. Read volumes of precipitates. Con- struct straight line curve with results from tubes 1 and 2. Locate readings of tubes 3 (total protein) and 4 (albumin) on curve. Read per- centages of protein. Globulin equals total protein less albumin. |
| Tube 2 | | Add 0.1 cc standard serum | |
| Tube 3 (total protein) | | Add 0.2 cc unknown serum | |
| Tube 4 (albu- min) | | Add 0.4 cc supernatant from centrifuged mix- ture of equal parts of unknown serum and saturated ammonium sulfate | |

Duplicate tubes 3 and 4 to the capacity of the centrifuge for determinations of the total protein and the albumin of additional serums. Omit tube 4 for determination of total protein alone and duplicate tube 3 with additional serums to the capacity of the centrifuge. Repeat tubes 1 and 2 with each run of the centrifuge.

This procedure provides a rapid and reliable method for the estimation of blood proteins. Except for the Bauer-Schenck centrifuge tubes, which are available commercially, no equipment is required other than that usually found in clinical laboratories.

SUMMARY

The method described for determining serum proteins depends on measuring the volumes of precipitated protein after the serum has been centrifuged in special graduated centrifuge tubes. Accuracy is obtained by the use of a standard serum to establish a curve of sedimentation characteristic of the conditions under which the determinations are made.

Notes and News

Army Institute of Pathology and American Registry of Pathology—What is now known as the Army Institute of Pathology was established in 1863 as the Army Medical Museum. During World War II the activities of the institute were greatly expanded, especially in the field of diagnostic pathology and research. There are now on file over 170,000 accessions. The results of research at the institute during the past few years will be published in a volume of about fourteen hundred pages as a part of the official history of World War II. The present director is Colonel J. Earl Ash, who will be succeeded on October 1 by Colonel Raymond O. Dart.

On request of Major General Norman T. Kirk, the Surgeon General of the Army, the Committee on Pathology of the National Research Council, Division of Medical Sciences, in late 1945 prepared a report on the future development of the institute. The report has been approved by the Surgeon General and by the War Department.

The essential recommendations in this report are (1) that a new building of adequate size be constructed, (2) that the Army Institute of Pathology be organized in four divisions—Department of Pathology, Army Medical Illustration Service, Army Medical Museum and American Registry of Pathology—each headed by a competent specialist, (3) that the staff of the institute be drawn from both the commissioned ranks of the Army and from the civilian professions, (4) that a comprehensive educational and training program be undertaken, (5) that the vast store of material at the institute be used for research and (6) that the services in pathology in the veterans' hospitals be centralized at the institute.

The American Registry of Pathology founded in 1922 thus is, and will continue to be, an integral part of the Army Institute of Pathology. There were, Jan. 1, 1946, over 43,000 cases registered. To effectuate the new plans as they relate to the registry, the National Research Council Division of Medical Sciences appointed a committee on the American Registry of Pathology. The members of the committee are Howard T. Karsner, chairman, Cleveland, Colonel J. E. Ash, Washington, Brigadier General George R. Callender, Washington, Colonel Balduin Lucke, Philadelphia, Robert A. Moore, St. Louis, Benjamin Rones, Washington, A. R. Shands Jr., Wilmington, Del., and Henry A. Swanson, Washington.

At the present time there are fourteen registries as a part of the American Registry of Pathology. These include Registry of Ophthalmic Pathology, established in 1922, sponsored by the American Academy of Ophthalmology and Otolaryngology, Lymphatic Tumor Registry, established in 1925, sponsored by the American Association of Pathologists and Bacteriologists, Bladder Tumor Registry, established in 1927, Kidney Tumor Registry, established in 1940, and Prostatic Tumor Registry, established in 1943, sponsored by the American Urological Association, Registry of Dental and Oral Pathology, established in 1933, sponsored by the American Dental Association, Registry of Otolaryngological Pathology, established in 1935, sponsored by the American Academy of Ophthalmology and Otolaryngology, General Tumor Registry, established in 1937, sponsored by the American Society of Clinical Pathologists, Registry of

Dermal Pathology, established in 1938, sponsored by the American Academy of Dermatology and Syphilology, Chest Tumor Registry, established in 1942, sponsored by the American Society of Thoracic Surgeons, Registry of Neuropathology, established in 1943, sponsored by the American Association of Neuropathologists, Registry of Orthopaedic Pathology, established in 1943, sponsored by the American Academy of Orthopaedic Surgeons, Registry of Veterinary Pathology, established in 1944, sponsored by the American Veterinary Medical Association, and Registry of Gerontology, established in 1945, sponsored by the Gerontological Society, Inc

Plans for additional registries are under consideration. A professional scientific society wishing to sponsor a registry should communicate with the Director, Army Institute of Pathology, 7th Street and Independence Avenue, S W, Washington 25, D C. The society appoints a committee to work with the director in supervision of the activities of the registry, and makes an annual contribution to the budget, which is administered by the National Academy of Sciences.

All specimens in the registry are made available for review and research to competent investigators. Sets of slides and accompanying syllabuses on special field are available for loan to the civilian professions and officers of the federal services. Physicians, dentists and veterinarians are urged to send an unusual specimen together with an abstract of the history to the registry. The contributor receives a report on each specimen and is asked to keep the registry informed of the results of follow-up examinations of the patient.

With the reorganization of the Army Institute of Pathology to be completed during 1946 and 1947, a full time scientific director of the American Registry of Pathology will be appointed and sufficient clerks and technicians will be available to assure adequate use of the registries for diagnosis, research, training of young men and education of the professions.

Books Received

EXPERIMENTAL HYPERTENSION BEING THE RESULTS OF A CONFERENCE ON THIS SUBJECT HELD BY THE SECTION OF BIOLOGY OF THE NEW YORK ACADEMY OF SCIENCES, FEBRUARY 9 AND 10, 1945 Special Publications of the New York Academy of Sciences, volume 3, pages 1 to 180 Price \$3.75 New York New York Academy of Sciences, 1946

This volume contains the papers read at the conference mentioned, with full reports of the discussions. The papers read are "Introductory Lecture on the Production and Pathogenesis of Experimental Hypertension," by Harry Goldblatt "The Mechanism of Renal Hypertension," by Eduardo Cruz Coke "Renin and Renal Hypertension," by L. F. Leloir "The Problem of the Occurrence of Vasoconstrictor Substances in the Peripheral Blood of Hypertensive Patients and Dogs After Injection of Renin," by Irvine H. Page, who also writes the preface "Experimental Chronic Hypertension Its Mechanism and Amelioration by Use of Various Blood Pressure-Reducing Substances," by Arthur Grollman "Treatment and Prophylaxis of Experimental Renal Hypertension with Renal Extracts and Marine Oils," by G. E. Wakerlin and others "Some Physiological Aspects of the Blood Pressure-Lowering Effect of Tissue Extracts in the Hypertensive Animal," by John W. Remington "A Change of Mechanism in the Course of Hypertension of Renal Origin," by Eric Ogden and others "Experimental Renal Hypertension and Amino Acid Metabolism in the Kidney," by Richard J. Brigg.

A summary of the facts presented is made by William Goldring, who also writes the introduction and (with H. Chasis and H. W. Smith) submits a statement on the question of a similarity of pathogenesis of experimental renal hypertension and human hypertension.

The book reveals that experimental hypertensive research is in "a healthy state and one rich with promise." It is a highly important contribution.

AUTOPSY DIAGNOSIS AND TECHNIC By Otto Saphir, M.D., pathologist, Michael Reese Hospital, and professor of pathology, University of Illinois Medical School, Chicago. Foreword by Ludvig Hektoen, M.D. Second edition, revised and enlarged. Pp. 405, with 69 illustrations. Price \$5. New York and London: Paul B. Hoeber, Inc., 1946.

The revision has increased the usefulness of this valuable book. Diseases of the breast are now described with the care and the detail (11 pages) which their importance demands. Several new chapters have been added: autopsies on still-born and other infants, anatomic findings in vitamin deficiencies, notes on the anatomic changes in certain tropical diseases, the nose and accessory sinuses. Certain chapters and tabulations have been enlarged—e.g., the chapter on unexpected death from natural causes, with notes on accidental death. The chapters on the autopsy permit, general technical considerations and the external examination are of special value.

PEPTIC ULCER ITS DIAGNOSIS AND TREATMENT By I. W. Held, M.D., attending physician, Beth Israel Hospital, and clinical professor of medicine (retired), New York University College of Medicine, New York, and A. Allen Goldbloom, M.D., assistant clinical professor of medicine, New York Medical College and Flower and Fifth Avenue Hospitals, associate physician, Beth Israel and Metropolitan hospitals, and associate cardiologist, Beth Israel Hospital, New York. Pp. 382, with 110 illustrations. Price \$6.50. Springfield, Ill.: Charles C. Thomas, Publisher, 1946.

ARTIFICIAL SUNLIGHT TREATMENT IN INDUSTRY A REPORT ON THE RESULTS OF THREE TRIALS—IN AN OFFICE, A FACTORY AND A COAL MINE By Dora Colebrook. Medical Research Council, Industrial Health Research Board, Report no. 89. Pp. 64. Price 30 cents. London: His Majesty's Stationery Office (New York: British Information Service), 1946.

ENVIRONMENTAL WARMTH AND ITS MEASUREMENT A BOOK OF REFERENCE PREPARED FOR THE ROYAL NAVAL PERSONNEL RESEARCH COMMITTEE OF THE

MEDICAL RESEARCH COUNCIL By T Bedford Medical Research Council War Memorandum no 17 Pp 40 Price, including charts for the calculation of environmental warmth, 2s 3d London His Majesty's Stationery Office, 1946

ARQUIVOS DO INSTITUTO DE PATOLOGIA GERAL DA UNIVERSIDADE DE COIMBRA Edited by Prof Melico Silvestre and Prof Mario Trincao Volume 30 Pp 488 Coimbra, Portugal, 1945

The volume contains a report of 469 pages on new serologic aspects of typhoid by Henrique de Oliveira

THE ROCKEFELLER FOUNDATION ANNUAL REPORT, 1945 Pp 346 New York Rockefeller Foundation, 1946

FORTY-THIRD ANNUAL REPORT, 1945-1946, IMPERIAL CANCER RESEARCH FUND, APRIL 1946 Pp 41 London Royal College of Surgeons, 1946

THE PRINCIPLES AND PRACTICE OF TROPICAL MEDICINE By L Everard Napier, Companion of the Order of the Indian Empire, Fellow of the Royal College of Physicians of London, formerly director and professor of tropical medicine, Calcutta School of Tropical Medicine Pp 917, with 195 illustrations Price \$11 New York The Macmillan Company, 1946

The objectives of this book are " to give an accurate and concise account of the more important tropical diseases from the points of view of epidemiology, aetiology, pathology, symptomatology, diagnosis, prevention, treatment and prognosis and to discuss in a general way such relevant subjects as methods for mitigating the effects of atropical climate, nutrition and anaemia in the tropics, and snakes and snake-bite" The book is planned for the student, the physician and the public health worker and is to be regarded rather as a textbook than as a book of reference The author is well qualified by long experience, especially in India, to write authoritatively

The book includes discussions of most of the tropical diseases and some that are not strictly tropical, such as tularemia, brucellosis and rabies The author deliberately omits some diseases commonly found in the tropics, such as typhoid, tuberculosis, smallpox, most of the neurotropic viruses and the systemic mycoses, because they are described in textbooks of general medicine He gives brief discussions of Rift Valley fever, melioidosis, paraspriue, hill diarrhea, gnathostomiasis, lathyrism, infantile cirrhosis of the liver, anemia in the tropics, myiasis and some other topics not mentioned in all books on tropical diseases The diseases with diarrhea are brought together, regardless of causes, into a section entitled "The Intestinal Fluxes"—an organization not commonly used, but useful nevertheless The longest chapter (67 pages) is devoted to malaria

This book is written from a practical point of view The material is well organized, and the writing is clear and concise The publisher has done an excellent job in printing, so that the headings and sections stand out distinctly The historical, epidemiologic and geographic approaches are excellent There are numerous good figures and 3 excellent plates in color, but photographs are not numerous and are of variable quality, probably because of the rough paper Photomicrographs, for the same reason, are poor (e g, figs 167 and 168, and the photomicrographs on plate II), a point of importance to pathologists The sections on pathology are generally briefer than most pathologists would like Laboratory diagnostic procedures include those which the author has found useful The bibliographies are short and are not intended to be exhaustive

This book is up to date on most points, but it does not fully reflect the experiences of World War II with respect to tsutsugamushi, DDT as used in the control of mosquitoes and flies, the control of filariasis by taking advantage of the short flight range of infected mosquitoes, and the number of relapses of patients with *Plasmodium vivax* malaria from the Southwest Pacific These criticisms are of minor importance. The book has so many excellent points that it stands out as one of the best books in one volume on tropical medicine The author has admirably achieved his objectives, and the book can be highly recommended

NECROPSIES ON OKINAWANS

Anatomic and Pathologic Observations

COMMANDER P E STEINER, MC(S), USNR *

INTRODUCTION

THE NECROPSIES reported here as a contribution to racial and geographic pathology were done on Okinawans during the military campaign on Okinawa Shima. They were done to establish the causes of deaths, to obtain information on transmissible diseases borne by the natives which might endanger United States personnel and to collect data which might help to establish a base line from which any deviation induced in the incidence of diseases by the American occupation might be measured. Of special interest was the incidence of schistosomiasis, leishmaniasis, malaria, filariasis, yaws, leprosy, the acute enteric diseases tsutsugamushi and Japanese encephalitis B. It became apparent early in the survey that some of the advance information on diseases occurring in the islanders needed modification. In general, health conditions were better than had been anticipated. During these necropsies some striking anatomic and pathologic differences from Americans and Europeans were observed, and they form an important part of this report. Such observations were of special interest when they suggested clues to the etiologic agents of the diseases concerned. The low incidence of disorders of the endocrine system and of degenerative diseases was especially impressive.

These examinations were made at a military government hospital from June 13 to July 30, 1945, inclusive. The patients had been evacuated from the front as it advanced to the southern end of the island. The majority were war casualties, but there were some with diseases not due to combat. They had been ill for periods ranging from several days to four weeks prior to being admitted to this hospital. Consequently, injuries which are soon fatal were seldom seen. On the other hand, most of the survey was made so soon after the campaign began that the full late effects which follow dislocation of a population by war had not yet developed.

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This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the United States Navy. The opinions and views set forth in this article are those of the writer and are not to be considered as reflecting the policies of the Navy Department.

The racial origins of the Okinawans, never having been recorded, are obscure. It is believed that to the original Ainu (Caucasoid) people, found here as in the Japanese Islands, were added, many centuries ago, Chinese, Korean, Japanese and to a lesser degree Malay strains by invasion and immigration. There may be minor differences in origin of the people on different parts of the island, just as there are differences in culture and language (three dialects). The people reported on here came from the southern half of the island.

One hundred and fifty necropsies were performed, of which 51 were on males and 99 were on females, a ratio of 1:2. In age the subjects varied from 18 days to 95 years. All ages and sexes were well represented except that there was a relative paucity of men in the military age groups, they having forcibly or voluntarily joined the Japanese military forces.

TABLE 1—*Distribution by Age and Sex*

| Age, Yr | Males | Females | Total |
|---------|-------|---------|-------|
| 0-1 | 6 | 4 | 10 |
| 2-5 | 6 | 10 | 16 |
| 6-10 | 2 | 8 | 10 |
| 11-15 | 6 | 10 | 16 |
| 16-20 | 4 | 12 | 16 |
| 21-30 | 4 | 9 | 13 |
| 31-40 | 3 | 11 | 14 |
| 41-50 | 6 | 9 | 15 |
| 51-60 | 4 | 11 | 15 |
| 61-70 | 7 | 12 | 19 |
| 71-80 | 3 | 2 | 5 |
| 81-90 | 0 | 0 | 0 |
| 91-100 | 0 | 1 | 1 |
| Total | 51 | 99 | 150 |

Forty persons (26 women and 14 men) were over 50 years old, a point of importance when one comes to consider the incidence of neoplastic and degenerative diseases. Additional data on age and sex are presented in table 1.

ANATOMIC OBSERVATIONS

The Okinawans are small, dark, Oriental people with good development and configuration of the body. Although they are usually classified as mongoloid, the implied resemblance is not pronounced. The epicanthic fold is generally inconspicuous or absent. And although they are classified with the yellow peoples, they are nearly all dark brown, probably due to Malayan blood and to prolonged exposure to the tropical sun, the majority being agricultural, rural and fisher folk. Rarely a person is seen who has a light skin but who otherwise resembles his neighbors. Such color is attributed by the Okinawans to European rather than to Ainu blood.

The average length of 26 men over 20 years of age was 153.8 cm (60.5 inches), the minimum was 142 cm and the maximum was 167 cm. The average length of 48 women of corresponding ages was 141.8 cm (55.8 inches), with a minimum of 126 cm and a maximum of 155 cm. The children were small so that at first there was a tendency to underestimate their ages by several years.

Body weights were not taken, but they appeared to be in good relation to the heights. Both sexes were well proportioned and muscular. Obesity was practically nonexistent, but many young women were plump. The thickest portion of the fat of the abdominal wall was generally under 10 mm in women, it was rarely over 15 mm. In men the fat, subcutaneous and internal, was even less abundant.

The carriage was erect, and in the women, who commonly carried heavy burdens balanced on their heads, the gait was graceful. Both thorax and pelvis appeared to be relatively flat in the anteroposterior diameter. The legs were usually straight, and when they were not, they were slightly bowed, they were muscular and rather short. The feet were well developed and broad across the toes. The great toe was well developed and it was usually separated from the second toe. Shoes were generally not worn, and toes were used for grasping in the weaving of ropes, baskets, and hats and in other handicraft.

In facial features the Okinawans varied between two extremes. Some were people with broad faces, thick lips, flat concave noses, rather thick skin and stocky bodies. Others had more delicate features, lower cheekbones, thin lips, thin, straight noses and slender bodies.

The hair was black, straight and soft and was less abundant on the average than that of Americans. In men it was generally moderately abundant on the scalp, the face, the axillas and the pectoral and pubic regions and scanty on the extremities. In women it was abundant on the scalp but scanty elsewhere, including the pubic region and the axillas. Hirsutism of the face, the arms and the legs was rare in women. The hair remained black into advanced ages, white hairs were rarely present in the early fifties, and practically all hairs remained black in some persons stated to be more than 70 years old. Baldness was rare.

The excellent muscular development of both sexes was apparent at a glance, but it was even more impressive on dissection. The pectoral and other muscles about the shoulders, the sternocleidomastoid muscle, the strap muscles about the thyroid gland and the other muscles of the neck, as well as the muscles of the extremities, and those about the spine, were exceedingly well developed, especially in the women. This was not surprising to any one observing the women digging in the fields or carrying heavy burdens on their heads as they trotted

along the paths and highways. Despite repeated childbirth, the abdominal wall tended to be flat and firm. Cystocele was observed in the perineum only twice and rectocele only once.

Internally, except for the small size of the viscera in conformity with the small body size, the most striking anatomic differences from Americans were found in the colon, the spleen, the pancreas and the biliary system. The colon was large, long and curiously tortuous. The cecum and the ascending colon were in the usual position, but at the hepatic flexure there was usually an S-shaped (S lying on its side) double curvature, with the three limbs of the S bound together by mesenteric folds. The center of the transverse colon lay low, usually sagging into the lower part of the abdomen. The splenic flexure, the descending colon and the rectum were normal, but the sigmoid flexure formed a long, large loop which frequently reached far into the right side of the abdomen. The explanation of this large, long tortuous colon was not certain. It was obviously an inherited characteristic, for it was already present in infants, in whom, however, it was less pronounced than in adults. The suggestion is made that it represents an adaptation to centuries of a predominantly vegetarian diet with its bulky fecal residue, analogous to that of herbivores.

Two noteworthy anatomic peculiarities were found in the biliary system. The gallbladder occasionally showed the fold and thickening of the distal end sometimes called "monk's hood." The common and hepatic bile ducts were frequently conspicuously larger than those of Caucasoid peoples. There was no evidence that this was due to disease.

The spleen was normally small. This reduction in size was out of proportion to that of the other viscera and to that of the body in general. On the other hand, the pancreas was relatively enlarged. It was firm and pale yellowish pink. Adipose infiltration like that commonly seen in the pancreas of Americans after middle age was seldom present, and the size, the color and the turgor were maintained into advanced ages. In adults of the United States the normal spleen is heavier than the pancreas, but in these Okinawans the normal pancreas was about one-third heavier than the spleen because of the relative enlargement of the former and the reduction of the latter.

CAUSES OF DEATH

Death was due to combat injuries in 100 cases, to noncombat (chiefly medical) causes in 36 and to a combination of war injuries and medical conditions in 14.

The combat injuries that caused death of 100 persons can be classified as follows: gunshot and shrapnel wounds, 88 persons; burns,

3, effects of inhaling chemical fumes, 9 Some additional persons who died of other causes showed nonfatal injuries of the three types mentioned, 14 had nonfatal gunshot and shrapnel wounds, 8 nonfatal burns and 1 showed effects of inhalation of chemical fumes

Of the 88 persons with fatal gunshot and shrapnel wounds, over half (45) had multiple wounds The number of such wounds per person is given in table 2 The number of major injuries borne by these people was at times astounding

TABLE 2—*Number of Gunshot and/or Shrapnel Wounds per Person in Eighty-Eight Fatal Cases*

| Wounds per Person | Cases |
|-------------------|-------|
| 1 | 43 |
| 2 | 19 |
| 3 | 8 |
| 4 | 5 |
| 5 | 3 |
| 6 to 10 | 5 |
| 11 to 25+ | 5 |

In table 3 are given the locations of the fatal gunshot and shrapnel wounds in 88 persons and the ultimate cause of death in these persons The chief cause of death was infection of wounds, usually with generalization of the infection or its toxic products A specific type of infection,

TABLE 3—*Location of Gunshot and/or Shrapnel Wounds in Relation to Cause of Death in Eighty-Eight Cases*

| Cause of Death | Cases with Given Location of Wound | | | | | Total |
|---|------------------------------------|--------|---------|-------|-------------|-------|
| | Soft Tissues | Thorax | Abdomen | Skull | Other Bones | |
| Toxemia, septicemia, pyemia, empyema or abscess | 10 | 3 | 4 | 4 | 14 | 35 |
| Tetanus | 10 | 1 | 0 | 0 | 20 | 31 |
| Hemorrhage | 3 | 2 | 4 | 0 | 0 | 9 |
| Gas gangrene | 3 | 0 | 0 | 0 | 4 | 7 |
| Pneumonia | 0 | 1 | 0 | 1 | 2 | 4 |
| Shock | 0 | 0 | 0 | 0 | 1 | 1 |
| Urinary retention | 0 | 0 | 0 | 0 | 1 | 1 |
| Total | 26 | 7 | 8 | 5 | 42 | 88 |

namely tetanus, was fatal in 31 persons and was present in a recovery stage in an additional one Shock and hemorrhage stand low as causes of death in this series because most of the patients evacuated to this hospital had been injured several days or even several weeks before admission The wounds were usually in poor condition when the patients entered the hospital, many teemed with maggots

The causes of death in 36 noncombat cases were as follows

| | |
|-----------------------------------|----|
| Malnutrition and pneumonia | 10 |
| Pneumonia (lobar 2, bronchial 4) | 6 |
| Tuberculosis | 5 |
| Dysentery (amebic 4, bacillary 1) | 5 |
| Cirrhosis of the liver | 2 |
| Toxemia of pregnancy | 1 |
| Typhoid | 1 |
| Heart disease (beriberi?) | 1 |
| Senility | 1 |
| Prematurity and fetal atelectasis | 1 |
| Skull fracture | 1 |
| Operative (noncombat) sequels | 1 |
| Uncertain | 1 |

Although they are classified as noncombat cases of death, the accuracy of this classification could be challenged, since in a number of them, no doubt, death was indirectly due to the war. This is particularly true of cases of death from malnutrition and pneumonia, death from these causes was observed predominantly in infants and children, some of whom had a history of existence in caves under adverse conditions. There was, however, no way of evaluating this factor more accurately.

The unfavorable influence of an injury on an infection, and vice versa, is illustrated by the 14 cases in which a war injury and a noncombat condition combined to cause death. These cases were as follows:

| | |
|---|---|
| Gunshot or shrapnel wounds and dysentery | 7 |
| Gunshot or shrapnel wounds and tuberculosis | 5 |
| Gunshot or shrapnel wounds and filariasis | 1 |
| Burns and tuberculosis | 1 |

CONGENITAL MALFORMATIONS AND ANOMALIES

Although congenital defects seemed to be more common than in America, only one, horseshoe kidney, was of clinical importance. In addition to the small size of the spleen previously mentioned, this organ showed a high incidence of multiple congenital clefts. Observations were recorded in 111 cases, in 2 of which no clefts of the spleen were seen, in 109 cases the spleen had from one to nine clefts, as follows: in 32, two clefts, in 24, three, in 18, four, in 11, five, in the remainder (24 cases), from six to nine. The clefts were usually shallow, but occasionally they were so deep as to nearly subdivide the organ. Accessory spleen, which probably represents a further degree of this malformation, was common. It was observed in 17 of 150 necropsies. In 4 cases two accessory splenules were seen.

Horseshoe kidney, hypoplasia of a kidney and double ureters were each found once. In 2 cases a diverticulum of the urinary bladder was encountered.

Abnormal numbers of pulmonary lobes were fairly common. One right lung had four lobes, and five right lungs were bilobed. Two left lungs were unlobed. In 1 case a lung showed multiple cysts, probably congenital.

The main peculiarity seen in the alimentary tract was the enlargement and tortuosity of the colon previously described. Meckel's diverticulum and a diverticulum of the jejunum were seen in 1 case each, and diverticula of the duodenum in 3 cases. Pseudodiverticula of the colon, so common in some parts of the world as acquired abnormalities, were not found in these Okinawans. Many persons with harelip and crossed eyes were seen in passing through the villages, but statistics on the incidence of these anomalies are not available.

One undescended testis was seen, and one axillary mammary gland. The ovaries were occasionally greatly elongated and cylindric, although small.

Two hearts showed patent foramen ovale. One liver had multiple cysts.

INTESTINAL HELMINTHIC INFECTIONS

Fifty-three persons had no recognizable intestinal helminths, but 97 (64.7 per cent) had one or more types. *Ascaris* was found in 67 of these persons (44.7 per cent), hookworm in 52 (34.7 per cent), and *Enterobius* and an unclassified tapeworm in 1 each. The incidence of infection was somewhat greater than is indicated by these figures, especially with respect to the small types of worms, as is shown in the following paragraph.

Because some of the intestines showed postmortem color changes or because the worms were scarce, hookworm infection was sometimes not recognized. The magnitude of this negative error was determined by comparing in 72 cases the results of gross inspection for worms with those of microscopic examination of the feces taken from the lower part of the colon for ova (performed by Lieutenant (jg) T. W. Simpson [MC] USN). The deficiency in the gross diagnosis of hookworms was found to be over 10 per cent. Even greater negative errors were found with respect to the other small intestinal helminths during this microscopic check on the macroscopic necropsy observations. Thus, in the course of the 72 microscopic examinations of feces, ova of *Trichuris* were found thirteen times, ova of *Enterobius* twice and larvae of *Strongyloides* twice. The hookworms were most commonly found in the upper part of the jejunum, where they clung to the mucosa, but at times they were found from the duodenum to the colon.

Ascaris was found in 67 cases (44.7 per cent). They were most common in the small intestine, where they sometimes formed coiled masses, but they were seen in all parts of the alimentary tract, from the mouth to the anus. The largest worm measured was 32.5 cm. Their numbers varied from 1 to 50. These numbers are shown in table 4.

TABLE 4—*Number of Ascarids per Person in One Hundred and Fifty Cases*

| Ascarids per Person | Cases |
|---------------------|-------|
| 0 | 83 |
| 1 | 26 |
| 2 | 12 |
| 3 | 5 |
| 4 | 7 |
| 5 | 1 |
| 6 | 4 |
| 7 | 3 |
| 8 | 2 |
| 9 | 1 |
| 16 | 1 |
| 17 | 1 |
| 18 | 2 |
| 19 | 1 |
| 50 | 1 |

The diagnosis of ascariasis obtained by examining the alimentary tract for worms of *Ascaris* was found to be more reliable than that obtained by examining the feces for ova in the 72 cases previously mentioned in which this comparison was made. This is explained by the fact that many persons (26) had only one worm and that others had only two or three. In cases in which all worms were males or nongravid females, diagnosis by examining the feces for ova was not possible.

There were no known pathologic complications arising from the activities of any of these worms.

NEOPLASMS

Malignant tumors were not encountered in this series of 150 necropsies. This paucity of cancers may be significant in view of the fact that 40 of these people were over 50 years old.

Benign neoplasms, on the other hand, were fairly numerous. The following types and numbers were found:

| | |
|--|---|
| Lipoma (jejunum 4, adrenal gland 1) | 5 |
| Adenomatous polyp (colon) | 1 |
| Leiomyoma (stomach 2, ileum 2, duodenum 1) | 5 |
| Benign epithelioma of skin | 2 |
| Dermoid of ovary | 1 |
| Lymphoma of ileum | 1 |
| Uterine fibroids | 1 |
| Endometrial polyps | 2 |

The observation that only 1 uterus among 54 from women over 20 years of age had fibroids is noteworthy. One uterus had been removed for unstated causes, the patient bore the only laparotomy scar in the entire necropsy series

INFECTIOUS DISEASES

Tuberculosis—Advanced tuberculosis was seen in 11 persons. Three of these were males, and 8 were females. Their ages were from 3 to 50 years. The tuberculosis was predominantly pulmonary in 10, calcification of lymph nodes due to tuberculosis (*tabes mesenterica*) was present in 1, a child 3 years old. In 5 persons the tuberculosis appeared to be the sole cause of death, but in the remaining 6, although the tuberculosis was equally advanced, there were also significant gunshot or shrapnel wounds (in 5) or chemical and heat burns (in 1). The tuberculosis was always extensive. It was acute in all instances except 1, in which fibroplastic pulmonary scarring was found in addition to acute caseous tuberculosis and areas of tuberculous pneumonia. The examiner had not seen in the United States a series of 11 consecutive cases of tuberculosis with such acute and extensive lesions. The acuteness of the lesions together with the absence of fibroplastic processes strongly suggested that these infections were recently acquired. The extrapulmonic tuberculous lesions which were present in the pleura, the lymph nodes, the larynx, the lower intestine, the adrenal glands, the spleen and other organs in these cases were not different from those commonly seen in America in cases of acute advanced pulmonary tuberculosis. Disseminated miliary lesions were present in 2 instances.

Subclinical tuberculous infection, minimal or inactive, was found as an incidental lesion in 24 cases, both the primary type and the reinfection type were observed. The primary type occurred with pulmonary tubercles in 9 cases, with tubercles of tracheobronchial lymph nodes in 5 cases and with both in 3 cases, minimal active pulmonary tuberculosis of the reinfection type was noted in 4 cases. Twenty of the patients with subclinical infection were females, and 4 were males, 14 were over 50 years old.

The total incidence of tuberculous infection found in these necropsies was 23.3 per cent, of which 7.3 per cent was advanced and 16 per cent minimal or inactive. The latter figure would be considered low for postmortem examinations in the United States. No data on the relative number of positive tuberculin reactors are available, with which these anatomic data might be compared. It is interesting that two types of lesions which are considered to be evidence of past tuberculous infection and which are commonly seen in the United States were never or seldom seen in these Okinawans. Thickened, puckered scars in the apical pleura were never seen. Focal fibrous pleural adhesions in

relation to subpleural lesions were rarely found at the apexes of the lungs, although adhesions were common elsewhere

The inference from these two observations is that either the incidence of tuberculous infection was low among these people or the infection of some of them was so slight as to leave insignificant anatomic residues. On the other hand, when tuberculosis gained a foothold, as it did fairly often under war conditions, it tended to be acute and massive. This may be a course induced by the adverse environmental factors incidental to the war, such as malnutrition, overcrowding with strangers in caves, and physical exhaustion, or it might indicate a low racial immunity.

Tetanus—Thirty-two of these persons had tetanus. One recovered from tetanus but died of pulmonary tuberculosis. Thirty-one died of the tetanus, although 2 of them had nearly recovered from it only to succumb to complications, namely, pulmonary abscesses, which were probably the result of faulty deglutition during the stage of severe tetanus. Tetanus was found at ages from 2 to 62. Its distribution by sex was in the same proportion as that of the entire necropsy series. In all cases it was caused by gunshot or shrapnel wounds, which were single in 15 persons and multiple in the remainder. Three persons with tetanus had over 10 wounds each. Many of the wounds involved one or more bones, others were in soft parts only. The tetanus made its appearance between the sixth and the twenty-first day, inclusive, after injury. There was no relation between the location of the injury and the period of incubation of the tetanus. The time of survival after the clinical onset was usually between twenty-four and forty-eight hours. The minimal period of survival was twelve hours. One person made a complete recovery from tetanus in twenty days but died later of tuberculosis, another had nearly recovered in ten days, and a third had recovered by the eighteenth day except for bilateral foot drop. The last 2 persons succumbed to pulmonary abscesses.

A constant gross finding in persons who died of tetanus was an obviously infected wound without, however, massive suppuration. The changes in the viscera also presented a constant picture, but they were not pathognomonic of the disease since they resembled those found in other forms of acute toxemia. These changes included moderate to massive pulmonary hyperemia and edema, dilatation of the right ventricle, cloudy swelling of the heart, the liver and the kidneys, and slight acute hyperplasia of the spleen. The central nervous system grossly appeared normal in every case. The diagnosis of tetanus was made clinically and was confirmed by the gross pathologic changes and occasionally by culture of material taken from the wound.

Gas Gangrene—Seven patients died of gas gangrene which developed as a result of shrapnel or gunshot wounds. Another person with gas

gangrene of the foot survived amputation of the foot only to contract tetanus five days later and succumb to this infection. Gas gangrene occurred in both sexes and at ages ranging from 4 to 63 years. No unusual features were recognized in this disease.

Rheumatic Fever—The only recognized manifestation of rheumatic infection was observed in the hearts of 4 persons. In 2, a man aged 55 and a woman 42 years old, both of whom died of infected shrapnel wounds, minimal scarring of the cusps of the mitral valve and of the adjacent chorda tendineae was noted. In neither was there any change in the heart or elsewhere as evidence of functional effects. The third patient, a woman aged 20 who died of tetanus, had thickening and fusion of the mitral cusps, a thrombotic vegetation on one mitral cusp, thickening of the endocardium of the left atrium and slight hypertrophy of the right ventricle. Another woman, 55 years old, who died of extensive pneumonia, had a history of cardiac decompensation of one year's duration. She had high grade mitral stenosis and both stenosis and insufficiency of the aortic valve, small thrombotic vegetations on both valves and moderate cardiac hypertrophy.

There was good evidence that rheumatic endocarditis occurs in some of these people and that the cardiac lesions resemble those seen in the United States, but the incidence of this involvement remains to be determined, although it appears to be low.

Bacterial Endocarditis—Bacterial endocarditis was found in only 1 person, a woman aged 64, in whom peritonitis and pyemia with multiple abscesses of viscera developed following a shrapnel wound of the abdomen. On one cusp of the tricuspid valve a shaggy vegetation was found measuring 14 by 12 by 2 mm.

Syphilis—Evidence of syphilis was found in only 1 necropsy. A man aged 69 died one month after sustaining a large shrapnel wound of the thigh, which had become infected. His aorta showed typical syphilitic involvement with diffuse dilatation of the ascending portion of the arch. The aortic ring and valve and the coronary ostia appeared normal.

Filariasis—Death from filariasis was not demonstrated in this series of necropsies. The incidence of infection as indicated by microfilarias in the blood is known to be high among Okinawans, but elephantiasis is not common. Thirteen persons showed anatomic changes characteristic of filariasis. Twelve were men. All were over 50 years of age except 2 who were both 30. The commonest lesion, found eleven times, was chronic inguinal lymphadenitis, sometimes it was associated with similar changes in the femoral or the iliac lymph nodes. Hydrocele was usually present in these men, it was unilateral in 6 and bilateral in 4. In 1 man and 1 woman the inguinal lymphadenitis was associated with elephantiasis of both legs, of a moderate degree. One man had extensive

lymphangiectasis and lymphadenopathy of the periaortic abdominal and pelvic lymphatic system of unknown but possibly filarial cause

Dysentery—The acute ulcerative lesions of dysentery were seen in the terminal part of the ileum or in the colon or in both in 16 necropsies. In 5 cases the dysentery was thought to have been the cause of death, in 7 it was considered contributory to death, and in 4 it was thought to be an incidental finding. The etiologic classification of these cases, based in large part on the gross pathologic appearance of the bowel and corroborated by further laboratory studies in only a few cases, was as follows: amebic dysentery, 6 cases; bacillary dysentery, 5 cases; dysentery of uncertain cause, 5 cases.

Pneumonia—Pneumonia was seen in 47 necropsies. Primary lobar pneumonia was found twice, primary bronchopneumonia four times, secondary bronchopneumonia thirty-seven times and chemical pneumonia four times.

Fibrous Pleural Adhesions—Fibrous pleural adhesions were found in 81 persons (54 per cent). They were bilateral in 37, on the right side in 26, and on the left in 18. In extent they varied from focal presence of fibrous bands to total obliteration of the pleural cavity. The incidence was approximately equal in the two sexes. Adhesions were already present in 3 infants aged 2 years. The incidence increased sharply in children up to the age of 10 years; in persons more than 10 years of age it was 61.4 per cent, and in those more than 50 years of age it was 77.5 per cent. Adhesions were seldom found at the apexes of the lungs and, because subpleural tubercles were uncommon, they were not often found in relation to peripheral tubercles. The cause would appear to be other than tuberculosis.

Miscellaneous Infections—Typhoid was found in 1 person, a girl 8 years old.

No proof of gonococcal infection was found on gross examination in these necropsies. In males there was no urethral stricture, prostatitis, epididymitis or testicular atrophy. Women appeared to be remarkably free from infection of the uterine cervix despite the fact that many of them were multiparous. Chronic pelvic inflammatory disease was found only a few times, and then with little to suggest that it was gonococcal.

There was no postmortem evidence of malaria, and this was in agreement with the clinical and hematologic evidence in these people at this particular time. No malarial pigment was seen in the spleen, the lymph nodes or the liver, and there were no splenic enlargements, acute or chronic, not readily explainable by other causes. In fact, the spleens, as previously mentioned, tended to be amazingly small. Smears from some spleens were examined for malarial parasites and invariably disclosed none.

There was no evidence of Trematode, or fluke, infection of the blood, the liver, the intestine or the lungs

The only evidence suggestive but by no means diagnostic of leishmaniasis consisted of large areas of cutaneous depigmentation in 2 old men

Yaws, leprosy, tsutsugamushi and Japanese encephalitis B were not seen in these necropsies

RETROGRESSIVE, DEGENERATIVE AND SENILE CHANGES

Senility—The onset of senile changes in Okinawans is delayed until advanced ages, and the complete picture comes even later. The first bloom of youth is early lost, especially in women, but thereafter the aging process appears to be slow, and guesses as to their ages are inaccurate. In the necropsies the picture was complicated by the presence of some malnutrition consequent to the war, so that the turgor of tissues and the amount of subcutaneous fat were unreliable indexes of age. The following criteria were found to be more reliable: atrophy of skeletal muscle and especially of cardiac muscle, percentage of white hairs among the black, all regions of the body being taken into account, calcification of the costal cartilages, skeletal changes, arteriosclerosis, atrophic pulmonary emphysema and "barrel chest." Judged by these criteria, many of the people were remarkably well preserved in the late sixties. Good posture was, on the whole, maintained among the old people. A few showed external deformity at joints but in those whose joints were examined by dissection there were minimal corresponding skeletal changes.

Cardiovascular System—The relative freedom of these Okinawans from degenerative diseases of the cardiovascular system was amazing. Hypertensive heart disease and malignant nephrosclerosis were not seen. Sclerosis of the aorta was found in 7 bodies, the stated ages of which were 55, 60, 65, 70, 71 and 95 years. Only the 95 year old subject was a female. The severity of the sclerosis was graded from 1 plus to 2 5 plus (on the basis of 4 plus as a maximum). The sclerosis was chiefly fatty and hyaline, but one or more calcified aortic plaques were seen in 4 cases. The middle-sized arteries usually showed only tortuosity. Only 1 case of coronary sclerosis with calcification was found, in addition there were 5 cases of slight noncalcific coronary sclerosis. In no instance were any of the serious complications or sequels of arteriosclerosis seen in the heart, the brain or the kidneys.

The heart appeared remarkably well preserved at all ages. It rarely showed senile atrophy and then only at an advanced age and to a slight degree. In women up to 70 years of age the heart was usually large and healthy appearing. Infiltration of the myocardium, particularly of the right ventricle, by adipose tissue from the epicardium, which is so

common in older women in some parts of the world, was not seen at all in these Okinawans, in conformity with their lack of obesity elsewhere and their ability to perform hard physical work. Myofibrosis of a degree visible grossly was seen only twice. Once it was found in relation to a slightly sclerotic but not occluded coronary artery, and once it appeared as an isolated cardiac lesion. Cardiac hypertrophy of the hypertensive type was seen only once, then it was associated with toxemia of pregnancy. Coronary occlusion was not found in these necropsies.

Only 3 cases of heart disease of known clinical significance appeared in this series of cases. One patient was a woman suffering from toxemia of pregnancy, she died in acute cardiac failure seven days post partum after refusing to stay in bed. The second patient was a woman aged 55 who died of extensive lobar pneumonia after having suffered from progressive heart failure for about one year, due to chronic mitral and aortic stenosis and insufficiency of the rheumatic type. The third patient was a man aged 71 who for three months had advanced cardiac decompensation, from which he died. He had practically no arteriosclerosis, and the cause of the heart disease was obscure, although the possibility of beriberi was considered.

Respiratory Tract—Atrophic emphysema was seen occasionally in some of the older people. In its advanced stages it was accompanied by "barrel chest."

Gastrointestinal Tract—Acute gastric ulcers were seen in 2 women, aged 46 and 70 years, both of whom had bacillary dysentery and infected shrapnel wounds, which did not involve any bones or the head. Another woman, 35 years old, who had extensive second and third degree burns from a flame thrower had five gastric ulcers and one duodenal ulcer, all acute. These ulcers were incidental postmortem findings.

Most of the appendixes appeared comparatively normal. In no person had the appendix been surgically removed. One appendix was 1 cm long and terminated in a slender fibrous cord. Another was involved by an extensive acute ulcerative process of the ileum and the colon which had perforated, causing peritonitis.

The colons, except for the tortuosity, the acute ulcerative lesions and the few benign tumors previously mentioned, appeared normal. The fecal contents were usually soft and voluminous. In no case were multiple pseudodiverticula seen of the type so commonly found in the colons of people in the United States.

Liver—Fibrous peritoneal adhesions were often present on various surfaces of the liver without appreciable thickening of the hepatic capsule elsewhere.

Cirrhosis of the liver appeared to be a common disease. It was found in 2 men and 4 women, who were between the ages of 47 and 70. In 3

of these persons the cirrhosis was mild, coarse and irregular in type, in the other 3 it was advanced, producing portal obstruction and causing death of 2. In 2 persons the liver was of the multilobular, irregular type, it was enlarged in one and reduced in size in the other. One small liver showed advanced cirrhosis of a monolobular type, with little evidence of regeneration.

Biliary System—Remarkably little disease was found in the biliary system. The most striking abnormality was the enlargement of the ducts previously described, considered to be normal. Chronic cholecystitis with marked thickening of the gallbladder wall was seen once. A solitary smooth oval gallstone, 21 mm in greatest diameter, was found in a woman aged 61. Another woman, aged 56, had 18 small faceted stones, each about 7 mm in diameter. Cholesterosis of the mucosa of the gallbladder was seen three times.

Spleen—The spleen was normally small in these people. In senility and malnutrition it underwent atrophy in accordance with that of the other viscera. It showed acute hyperplasia in the presence of some infections, and chronic splenomegaly was observed in several cases of generalized chronic passive congestion and in cases of hypertension of the portal system associated with cirrhosis of the liver. No malarial pigmentation was recognized.

Lymphatic System—The most conspicuous and consistent change in the lymphatic system was hyperplasia of the mesenteric lymph nodes, present in about 90 per cent of these persons. It was more pronounced in the young than in the old. The degree of enlargement was usually 1 or 2 plus (on the basis of a maximum of 4 plus). The nodes were discrete, elastic and pale yellowish gray, there was no periadenitis. The cause of this mesenteric lymphadenitis was not determined, but it appeared to be related to the intestinal helminthic infections which were so common.

The tracheobronchial lymph nodes showed less than the expected incidence of enlargement, because of the low incidence of tuberculosis in these nodes. The inguinal, femoral and iliac lymphadenitis related to filariasis has been described with that disease.

Skeleton—Except for the traumatic injuries, no major pathologic conditions were seen in the skeletons of these people. The commonest retrogressive abnormality was softening of bones, noticed especially in the sternum and the ribs, so that they bent and fractured easily, although incompletely. This type of osteomalacia and osteoporosis was present thirteen times. It was found equally in both sexes and occurred after 50 years of age with two exceptions (30 and 44 years). It accompanied malnutrition, wasting diseases and senility. A differentiation between osteoporosis and osteomalacia was not made.

Osteoarthritis of vertebral bodies, so common in Americans over 50 years old, and characterized by a small amount of "lipping" in the lumbar or the thoracic regions, was seen only nine times and then as an incidental postmortem finding. It was always of a mild degree. All the subjects were over 50 years of age, and the sexes showed an equal incidence. Deforming arthritis of several joints of one hand was seen in a woman 52 years old.

The costal cartilages were not calcified even in the aged.

The teeth were generally good until middle age, after which they deteriorated rapidly. Young people frequently had gold crowns or half crowns on four upper front teeth, but these had been placed on sound teeth as an aid to beauty.

Endocrine System—The pancreas showed no recognizable pathologic changes. It was large, firm and pale yellowish pink. Even in the aged it failed to undergo atrophy, and there was little or no tendency toward adipose tissue infiltration and replacement. There were no known cases of diabetes in this series, and glycosuria was not seen in the clinical laboratory during this time.

The thyroid gland was small, pale tan to pale yellowish brown, firm and without any nodules except in 2 instances in which it showed small colloid cysts. Each person had only a few grams of thyroid tissue.

The thymus gland was not remarkable except that, because of the small amount of mediastinal fat, it was easily seen.

The adrenal glands were characterized by their large size in proportion to that of the body. They were large even in cases of severe malnutrition. They contained no visible areas of nodular cortical hyperplasia.

Generative Organs—The prostate gland was usually small and symmetric. Nodular hyperplasia was seen in only 2 elderly men, aged 65 and 73 years. The enlargement was slight in each and had not produced any obstruction.

The seminal vesicles and the testes presented no noteworthy changes.

The uterine cervix was remarkably free from lacerations, erosions and chronic infection despite known child bearing. Uterine fibroids were practically nonexistent, only 1 uterus, from a woman 43 years old, showing multiple small tumors. Two uteri contained small endometrial polyps. The fallopian tubes, except for several with cysts and adhesions, were not abnormal. The ovaries commonly had small cysts. The mammary glands were not large, but the glandular tissue was abundant and the adipose tissue scarce. No cystic disease was found, and inspissated secretion was never seen in the ducts.

Urinary Tract—Renal disease was extremely uncommon in these necropsies. One congenitally hypoplastic kidney was seen in a 9 month

old infant, but not a single contracted kidney, primary or secondary, was found. Coarse, retracted scars on the greater curvature, considered by some to follow arteriosclerosis and by others to represent pyelonephritic scarring, were seen only three times. Acute urinary retention, unilateral or bilateral and with or without infection, was the most common pathologic condition noted. It complicated injuries which involved the lower part of the urinary tract. Two cases of mild glomerulonephritis were recognized.

Calculi were found in the urinary tracts of 2 persons. One stone was found in the renal pelvis of a girl aged 9, who died of a shrapnel wound with an infected compound comminuted fracture of the femur and advanced pulmonary and miliary tuberculosis. A woman 42 years old, who died of exactly the same causes, had two yellowish brown concretions in one of the renal pelvis. They had been wounded five and six weeks, respectively, before death, and the woman, at least, is known to have been treated with sulfadiazine.

Tattooing—Tattooing of the backs of the hands as a form of branding at the time of marriage was practiced some years ago. The youngest woman showing these blue marks was 50 years old. Of 26 women over 50 years of age, 18 showed tattooing. It was seen in its most elaborate form in the very old women, in whom it sometimes extended from the distal phalanges of the fingers to the upper margins of the wrists. In its mildest form it consisted of blue dots up to 10 mm in diameter over the middle articulation of the great finger and the adjacent third finger of each hand.

MALNUTRITION

Malnutrition, of apparently recent origin, was seen a number of times and in all age groups. It appeared to be a factor contributory to death in a number of instances. In some cases there was a history of inadequate intake of food before admission to the hospital. However, the diet of these people prior to the invasion appears to have been excellent as judged by the absence of deficiency diseases, by longevity and by the stated and observed high fertility. It did not appear to promote rapid growth in the children, although they appeared well formed, had good teeth and were not pot bellied.

The diet formerly consisted mostly of carbohydrates, with sweet potatoes, rice and various vegetables predominating. The intake of fats was low, and the protein content of the diet appeared to have been deficient, although it probably actually was not. Proteins were chiefly vegetable in origin (soybeans). Meat was eaten infrequently, and little milk was used. Pork, fish and goat were the chief meats. Fruits were grown on a small scale. In the hospital the patients preferred their native diet of rice balls and sweet potatoes to American food, although

in all fairness it must be stated that they were judging the American diet largely by K rations

At necropsy the chief evidence of malnutrition apart from emaciation was edema. It was thought to be a nutritional edema because it occurred in persons who had normal-appearing hearts and kidneys, who had little or no evidence of passive congestion and who often had a history of recent inadequate intake of food. Furthermore, this type of edema, which was seen only a few times during the first weeks of this survey, underwent a marked increase during the last weeks. It was seen nineteen times—twelve times in the last 22 necropsies.

Lieutenant (jg) T. W. Simpson studied the blood plasma protein levels in nearly 50 Okinawans who had edema and ascites in the absence of demonstrable cardiac or renal disease. He found the plasma proteins to be as low as 2.5 Gm. per hundred cubic centimeters, with an average of 4.5 Gm. The patients with an advanced degree of edema and anasarca made a poor response, if any, to the protein added to the diet or even to injected serum albumin, but those with a milder degree of edema showed a good response to the dietary treatment. He also found that most of these patients had microcytic hypochromic anemia.

INFORMATION OBTAINED FROM OKINAWAN PHYSICIANS

Because the number of necropsies was small, the possibility existed that the sampling was not representative and that unwarranted conclusions were made, despite the fact that the findings were in agreement with those of Benjamin¹. Furthermore, certain nonlethal diseases, as well as those present on the island only intermittently, even though in severe epidemic form, might be overlooked in a quick survey of this type. Evidence of another type which might corroborate, refute or supplement that obtained at necropsy was therefore desirable. This was systematically obtained from native Okinawan physicians.

A questionnaire was prepared, which covered over a hundred diseases, with particular emphasis on incidence, etiologic factors and importance. In interviews these questions were put to eight Okinawan physicians, who had practiced for periods varying from eight to forty-one years. With one exception they had attended medical school in Japan. They were found to be well informed, especially with respect to the infectious diseases. They had had large practices in the following towns: Shuri, Naha, Itoman, Miyazato, Haenna, Awasi and Sashiki. Their answers were recorded during the interview. When answers were reviewed, it was found that their opinions on medical matters agreed remarkably well but that there was some lack of agreement in answers to questions asked at the same time with regard to other fields. Necropsies were formerly done only occasionally, on order of the police. In the prefectural hospital at

¹ Benjamin, E. L. U. S. Nav. M. Bull. 46: 495, 1946.

Naha they were done at the request of physicians. There is no religious prohibition of such examinations, but the great reverence in which the dead are held and worshipped discourages wide practice of necropsy.

So far as it could be compared, the information obtained by the two methods, namely, necropsy and questioning of physicians, was in agreement, with two notable exceptions. Nephritis, type undetermined, was stated to be common, especially in children and very aged persons, among whom, it was said, the mortality due to this condition was low. At necropsy, on the other hand, the kidneys showed little evidence of past infection or of vascular disease. The other outstanding discrepancy concerned the cases of dysentery. The physicians stated that in practically all cases dysentery was amebic, whereas at necropsy verified dysentery was always bacillary. It is of course possible that both findings are correct if it is assumed that bacillary dysentery showed a relative increase with the war.

The physicians agreed with each other and with the necropsy observations that the major fatal diseases were tuberculosis, dysentery and pneumonia. To these they added as important and common diseases diarrhea and gastroenteritis, hookworms and ascariasis, nephritis, epidemic conjunctivitis, scabies and occasionally epidemic colds, dengue and influenza.

They further agreed that the degenerative and retrogressive diseases were uncommon, rare or absent. Atherosclerosis was characterized as uncommon, as was hypertension. Both were thought to be more common in the cities, in old men, and sake drinking was thought by some to be an etiologic factor—a point of interest to those who believe that alcohol relaxes the arteries. Angina pectoris and coronary occlusion were said to be rare, but cerebral apoplexy was thought to be fairly common in old people. Cardiac failure and heart disease of all types were uncommon. Many people were said to reach 80 years of age, and a few lived to 100.

Cancers were said to be fairly common in old people. Their incidence was generally given in the following order: gastric, uterine, mammary, esophageal and lingual. The larynx, the penis and the liver were each mentioned only once as a site of cancer. Sarcoma was said to occur rarely and then only in bones and in the vagina. Leukemia had never been seen by most of these physicians.

Special attention was given to the infectious diseases, and some interesting answers were recorded. The following diseases were said not to occur on Okinawa: anthrax, botulism, glanders, schistosomiasis, typhus and yaws. Cholera formerly caused epidemics but had not been seen for over forty years. Plague was introduced once long ago into Naha, the principal seaport and capital, but was promptly stopped by quarantine. Smallpox was no longer present because every child was repeatedly vaccinated. Tsutsugamushi was said to be no nearer than Formosa.

Diseases described as rare included the following poliomyelitis, which is seen occasionally in the summer, scarlet fever, although they saw this disease commonly in Tokyo and elsewhere as medical students, rabies, which was present only rarely and then in Naha, the only place on the island where dogs were kept, rheumatic fever, the low incidence of which corresponds with that of scarlet fever. However, the incidence of rheumatic fever is probably not as low as these physicians believed, for endocarditis of rheumatic type was found in 4 hearts at necropsy.

Diseases described as uncommon included mumps, which many children were said to escape, bacterial endocarditis, diphtheria, which was found at all times of the year, the island being subtropical, meningitis, which was usually tuberculous and found chiefly in children, septicemia. Malaria was said to be formerly contracted only on the north end of Okinawa and on some of the adjacent islands. Syphilis was seen practically only in young men who had been with prostitutes in cities (Naha). Both chancres and gummas had been recognized, the latter on the legs and in the liver. Gonorrhea was commoner than syphilis, and it occurred under similar conditions.

Diseases said to be common included the following tuberculosis, which was most common in young adults of both sexes but occurred occasionally in younger or older persons. Several doctors volunteered the information that it was especially common in the young people who had worked in the big industrial cities of Japan, such as Osaka. Diarrhea was said to be common, especially in the summer. A small percentage of the patients had amebic dysentery, which was often fatal in infants and very aged persons. Bacillary dysentery, the physicians said, was rarely found in Okinawans. Measles and whooping cough were mild diseases that occurred in epidemics nearly every summer and autumn, and sooner or later every person was attacked. Pneumonia occurred commonly in all months and at all ages but especially in children under 10 years old, in whom it was often fatal. Tetanus was seen in farmers as a consequence of injuries and in newborn infants as a fatal infection of the umbilical cord. (Childbirth was usually attended by relatives or friends, less commonly by a midwife, and by a physician only in case of complications.) Ascaris and ankylostomiasis were described as common. The latter was said to be an infection predominantly of farmers. Sometimes it produced anemia in children. Infection by *Taenia saginata* was distinctly less common, and trichinosis was rare. Filarial infection was common, but elephantiasis was not a big problem. It occurred mainly on the southern and eastern end of Okinawa and on some of the outlying small islands. Scabies was a common infection of children before the war, but the widespread, heavy infestations with lice seen after the invasion and during these necropsies were a product of the war.

Certain diseases were placed in a special group because they occurred in epidemics at irregular intervals as follows. Dengue occurred at irregular intervals during the summer and was rarely fatal. Typhoid occurred irregularly in the summer, and the mortality was high. Encephalitis, believed to be Japanese encephalitis B, was a serious disease which occurred in small epidemics during the summer months at irregular intervals of a few years. Epidemic conjunctivitis occurred nearly every summer and every autumn in all age groups but predominantly in children. There were about a thousand lepers collected in a government leprosarium on a small outlying island. The incidence of this disease was stated to be decreasing.

It was the opinion of these physicians that there was little or no malnutrition or vitamin deficiency on the island. Anemia was present only in some cases of hookworm infection, in children. Pernicious anemia was said to be rare. Toxic goiter was rare. Diabetes mellitus was rare and was seen only in persons who were more than 40 years of age.

COMMENT

Apart from the combat injuries these necropsies reveal differences from what would be expected in a similar series in the United States. Some diseases were commoner, a few were rarer, and the evidence with respect to others was inconclusive. The diseases which were rarer are especially intriguing, because their study offers the possibility that the etiologic factors may be discovered.

The most striking finding was the low incidence of retrogressive and degenerative changes. Senility came late, and cardiac and skeletal muscles remained well preserved even in the aged. Arteriosclerosis also developed late and to a moderate degree or not at all, and its lethal sequels in the brain, the kidneys and the heart were never seen. Hypertensive cardiovascular-renal disease was not seen except for 1 case of the type associated with toxemia of pregnancy. In fact, heart disease of all types, including congenital, rheumatic, bacterial endocarditic, syphilitic, hypertensive and coronary arteriosclerotic, was uncommon or absent. Renal diseases of all types was rare. Although cirrhosis of the liver was common, disease of the biliary tracts, including cholelithiasis, was not. After the life of these people had been studied, two possible etiologic factors appeared outstanding in explanation of these observations. They were (1) a low tension, placid, although physically strenuous, life and (2) a simple, predominantly vegetarian diet.

The low incidence of malignant neoplasms, although expected, was also of interest. The explanation might lie in the relative absence of factors sometimes considered to be etiologically important. For example, stasis of ducts and cystic disease were not found in the mammary gland,

and the history of these women was typically one of numerous pregnancies with prolonged periods of lactation. The mammary glands of women past the teens appeared small and pendulous, but the glandular tissue was abundant and remained remarkably well preserved. These women rarely had erosions or infections of the uterine cervix. There was a suggestion of excellent endocrine balance in the absence or the low incidence of hirsutism in women, pathologic obesity, thyroid disease, diabetes, cystic diseases of the mammary gland, fibroids and endometrial disease of the uterus, and nodular hyperplasia of the prostate and the adrenal glands. Vitamin deficiencies with metaplasias in various epitheliums were probably absent. With respect to the alimentary tract, the diet was simple, bulky and predominantly vegetarian. There was no disease of the stomach except ulcers, these were acute and may have been related to the psychosomatic stress incidental to the bombing and invasion. There were no scars of healed peptic ulcers. Bowel habits are said to be excellent, testified to also by the absence of pseudo-diverticula of the colon. There were few polyps of the colon, and appendical disease was rare. The skin was deeply pigmented against the tropical sun, and there were no industrial hazards, as possible explanations for the absence of cancers of the skin and of the respiratory tract. Lithiasis of any location was uncommon.

Endocrinologically, these people appeared to be remarkably well balanced. They were highly fertile. Disease of the thyroid, parathyroid, pituitary and adrenal glands, the pancreas and the gonads was either nonexistent, rare or unrecognized. The uteri rarely had fibroids, and the prostates equally rarely showed benign adenomatous hyperplasia. There was no instance of pathologic obesity and no hirsutism in women. The thyroid glands were small and without nodules or adenomas, only 2 contained colloid cysts. These people had no acne vulgaris.

The most important medical problems apart from the combat injuries were the infectious diseases. Tuberculosis, dysentery, pneumonia and tetanus took heavy tolls. Filariasis appeared to be common but was of a benign form which led to little elephantiasis. The incidence of intestinal helminthic infections was high but here again the clinical effects were negligible. Many other diseases present in tropical countries and expected on Okinawa were absent.

Benjamin recently reported on a series of necropsies comparable to that presented in this paper. Except for a lower incidence of tetanus in his series, the results are in close agreement.

BILATERAL OVARIAN APLASIA

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AND

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NEWCASTLE UPON TYNE, ENGLAND

BILATERAL aplasia of the ovaries unassociated with gross congenital malformation allows an opportunity for a study of the part played by ovarian function in the development of the human female. Kermauner¹ in 1912 critically reviewed the literature and considered most of the reported cases to be of doubtful validity because of lack of detailed histologic examination. Since then 6 well authenticated cases in which the autopsy reports approach completeness have been recorded by Pich,² Rossle and Wallart,³ Graber,⁴ Olivet⁵ and Schurmann.⁶ To these Pich would add the rather more doubtful case of Randerath,⁷ though histologic examination was incomplete, and that of Priesel,⁸ in which pathologic destruction of the ovaries could not be excluded.

That this interesting and fundamental deficiency is not confined to the human subject but can be found throughout the whole vertebrate phylum is evidenced by the reports of its having occurred in fish and other animals (Kermauner¹), in the reptile *Lacerta* (Jacquet⁹), in the pigeon (Riddle¹⁰) and in the female rat (Beach¹¹).

The reported cases of aplasia of the ovaries of the human subject have many characteristics in common. The subject is recognizably female but shows no secondary sexual development, primary amenorrhea is present and underdevelopment of the external and the internal genitalia is found. In several of the cases the subject has been below

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1 Kermauner, F Beitr z path Anat u z allg Path **54** 478, 1912

2 Pich, G Beitr z path Anat u z allg Path **98** 218, 1937

3 Rossle, R, and Wallart, J Beitr z path Anat u z allg Path **84** 401, 1930

4 Graber, H Virchows Arch f path Anat **299** 80, 1937

5 Olivet, J Frankfurt Ztschr f Path **29** 477, 1923

6 Schurmann, P Virchows Arch f path Anat **263** 649, 1927

7 Randerath, E Virchows Arch f path Anat **254** 798, 1925

8 Priesel, A, in Henke, F, and Lubarsch, O Handbuch der pathologischen Anatomie, Berlin, Julius Springer, 1931, vol 6, p 1

9 Jaquet M Bibliog anat **3** 267, 1895, cited by Riddle¹⁰

10 Riddle, O Brit J Exper Biol **2** 211, 1925

11 Beach, F A Anat Rec **92** 289, 1945

the average height, and the term "sexogenic dwarfism" has been used in this connection by Rossle and Wallart³ Coincidental minor congenital abnormalities are frequent

One other case is now recorded, compared with the other reported cases and discussed in the light of modern theories of sex determination and development

REPORT OF A CASE

A white unmarried woman aged 35 years was admitted, April 6, 1944, under the care of Dr T H Boon She complained of painful swelling of the left leg and buttock of seven days' duration Previously she had always been pale and thin and had never menstruated Twelve months earlier she suffered from a "nervous breakdown" which lasted a few weeks Three months before admission the last symptoms recurred and in the ensuing two months she lost 10 Kg On admission she showed an edematous, reddened left leg and a swollen doughy abdomen There was complete absence of secondary sexual characteristics, the blood pressure was 130 systolic and 90 diastolic, and the urine revealed a trace of albumin

Roentgen examination of the lungs, April 11, showed an opacity of the left middle zone suggestive of tuberculosis (A previous examination, February 11, had shown no abnormality) The radiologist also reported that the sella turcica was small The red blood cells numbered 3,200,000 per cubic millimeter, and the hemoglobin content was 54 per cent (Sahli) The clinical diagnosis was abdominal and pulmonary tuberculosis and questionable Simmonds' disease Estimation of 17-ketosteroid excretion was planned, but the patient died April 12, before this could be carried out

Autopsy (April 14)—The length of body from vertex to heel was 165 cm The distance between the anterior superior iliac spines was 22.5 cm No pubic nor axillary hair was present, and the vulva showed only scanty hair The hair of the head and the eyebrows appeared normal The breasts were not developed beyond the infantile stage The skeletal development appeared normal, and all the epiphyses were closed

There was moderately active caseous tuberculous lymphadenitis affecting the cervical, mediastinal, portal, mesenteric and para-aortic lymph nodes A chronic tuberculous ulcer of the ascending colon had been causing chronic obstruction, and there was recent tuberculous peritonitis The tuberculous process had caused thickening of the broad ligaments which partially obscured the uterine tubes Recent tuberculous lesions were present in the lower portion of the upper lobe of the left lung Culture showed the tubercle bacilli to be of the human type

The kidneys were unequal in size, the right weighing 126 Gm and the left 54 Gm The right kidney, renal artery and ureter were normal in appearance The left kidney showed a slightly granular surface, and the width of the cortex was reduced to 0.2 cm The left renal artery bifurcated 1.5 cm from its origin and was not reduced in size The left ureter was normal in size

The external genitalia were definitely female but were not developed beyond the prepubertal stage The vaginal introitus was small and the wall smooth The uterus was infantile with a circular external os The cervix measured 2.5 cm and the body 2.3 cm in length, the ratio lying between that of the infantile and that of the adult uterus Both uterine tubes were recognized, they appeared thin and underdeveloped No ovaries were discovered, and no ovarian tissue was found in the usual ectopic sites

An antemortem thrombus was present in the left iliac vein, the left uterine plexus and the left femoral vein There was no pulmonary embolism

The aorta had a maximum circumference of 4.5 cm, and the wall appeared thin, there was no coarctation. The main blood vessels showed no abnormality.

The endocrine glands were grossly normal and will be described later with their histologic appearances.

The other organs showed no obvious abnormality, and there were no other developmental errors.

Histologic Examination—Uterine Tubes Serial blocks for histologic examination were cut along the whole length of each uterine tube at right angles to the long axis. Sections from these showed that each tube was the site of recent tuberculosis, which had nowhere destroyed the structure of the organ. The surrounding tissues showed many foci of tuberculosis with formation of giant cell systems, but everywhere the tuberculous lesion was of recent origin. There was no evidence of older tuberculosis or of other pathologic processes which might have destroyed the ovaries.

In the distal third of each mesosalpinx was found a collection of tubules which on first sight resembled epididymis (fig 1). These tubules were lined by an epithelium which varied from a low cubical to a high columnar type, with occasional ciliated cells. A few of the cells contained acidophilic granules, and the lumens of several of the tubules contained amorphous acidophilic material. One lining cell was seen in mitosis. Several of the tubules appeared to be dilated, and one or two were definitely cystic. Scanty smooth muscle fibers arranged concentrically could be made out in the walls of most of the tubules, but in several tubules muscle appeared to be absent. The tubules were arranged in loose coils in a delicate collagenous tissue. Most of these tubules were similar to the epoophoron found in this position throughout life. The epoophoron of the present subject has been compared with those of 6 women in the same age group who had undergone hysterectomy, and though more muscle is present in some of the control specimens, no essential structural difference has been found (fig 2, this control section shows the greatest difference observed). A hydatid of Morgagni was present showing no unusual characteristics. The duct of Gartner was not found.

Sections from one block showed in a dense collagenous stroma a collection of large cells with abundant acidophilic granular cytoplasm and round, slightly eccentric, deeply staining nuclei (fig 3). Some of these cells contained a few fine golden brown granules which were negative for the prussian blue reaction and resembled the pigment of brown atrophy. These cells were not in close relation to the epoophoron, they were indistinguishable from interstitial cells and were regarded as such.

No ovarian tissue, cortical or medullary, was found in any of the blocks of tissue.

Uterus The endometrium was thin and showed no evidence of estrogenic stimulation. The endometrial and cervical glands appeared scanty and underdeveloped.

Vagina The epithelium was thin, consisting only of three to four layers of squamous epithelial cells (fig 4). No glycogen was demonstrated, but in view of the interval of time between death and autopsy this may be of little significance.

Breast A hemisection of the organ taken through the nipple (fig 5) showed only a few underdeveloped ducts, the mouths of which were plugged with keratinized cells. No acini were noted. A considerable amount of muscle was to be seen in the nipple, but the latter hardly projected above the surrounding skin. The total thickness of breast tissue was less than 1.0 cm.

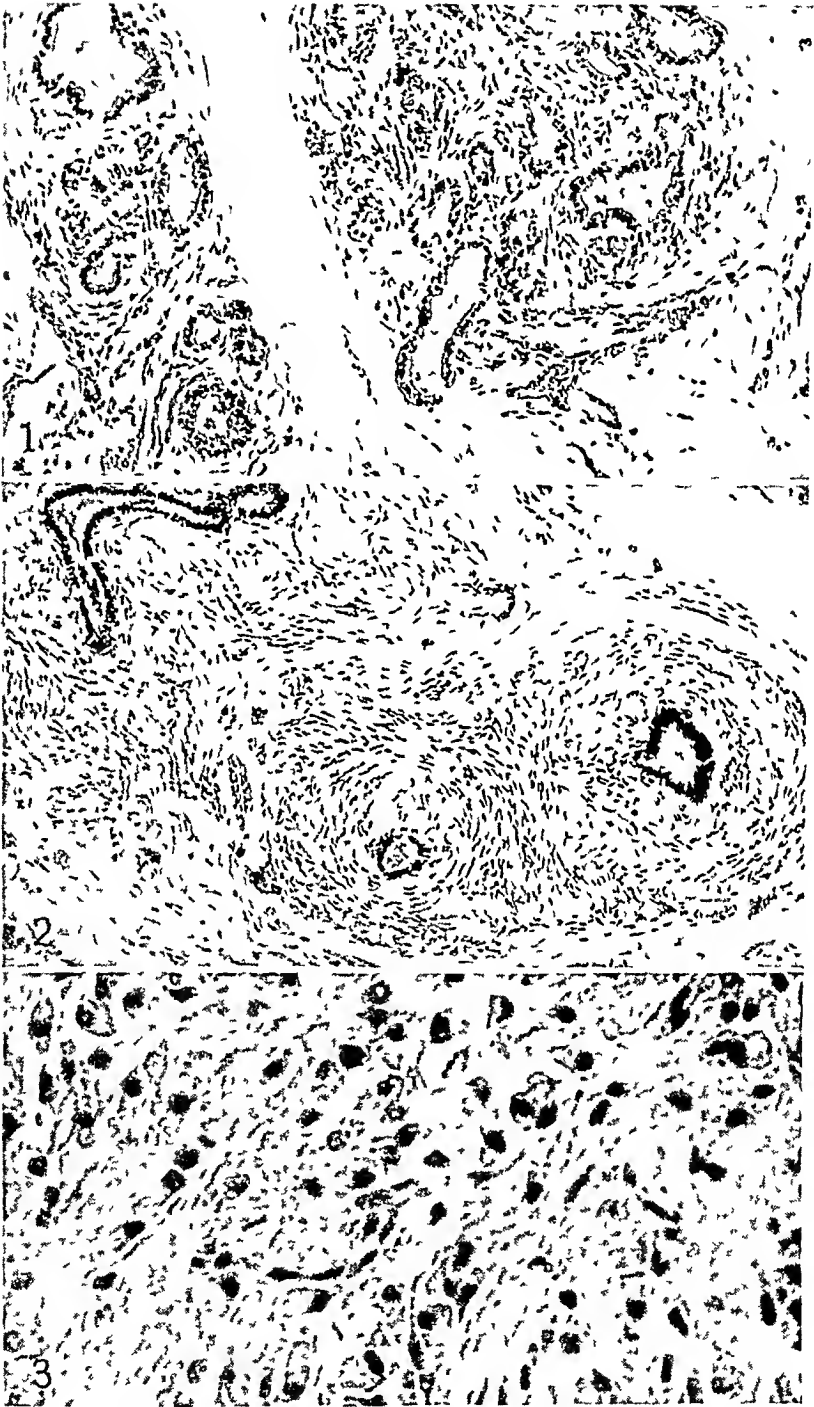


Fig 1—Epoophoron showing structure of tubules found in the mesosalpinx Hematoxylin and eosin, $\times 92$

Fig 2—Epoophoron from a woman aged 45 for comparison with that in figure 1 Hematoxylin and eosin, $\times 92$

Fig 3—Section showing interstitial cells in collagenous stroma Hematoxylin and eosin, $\times 480$

Adrenal Glands The combined weight was 12 Gm, and as the usual weight found with a comparable amount of dissection is in the region of 16 Gm, the glands must be considered small. Histologically they show no obvious abnormality.

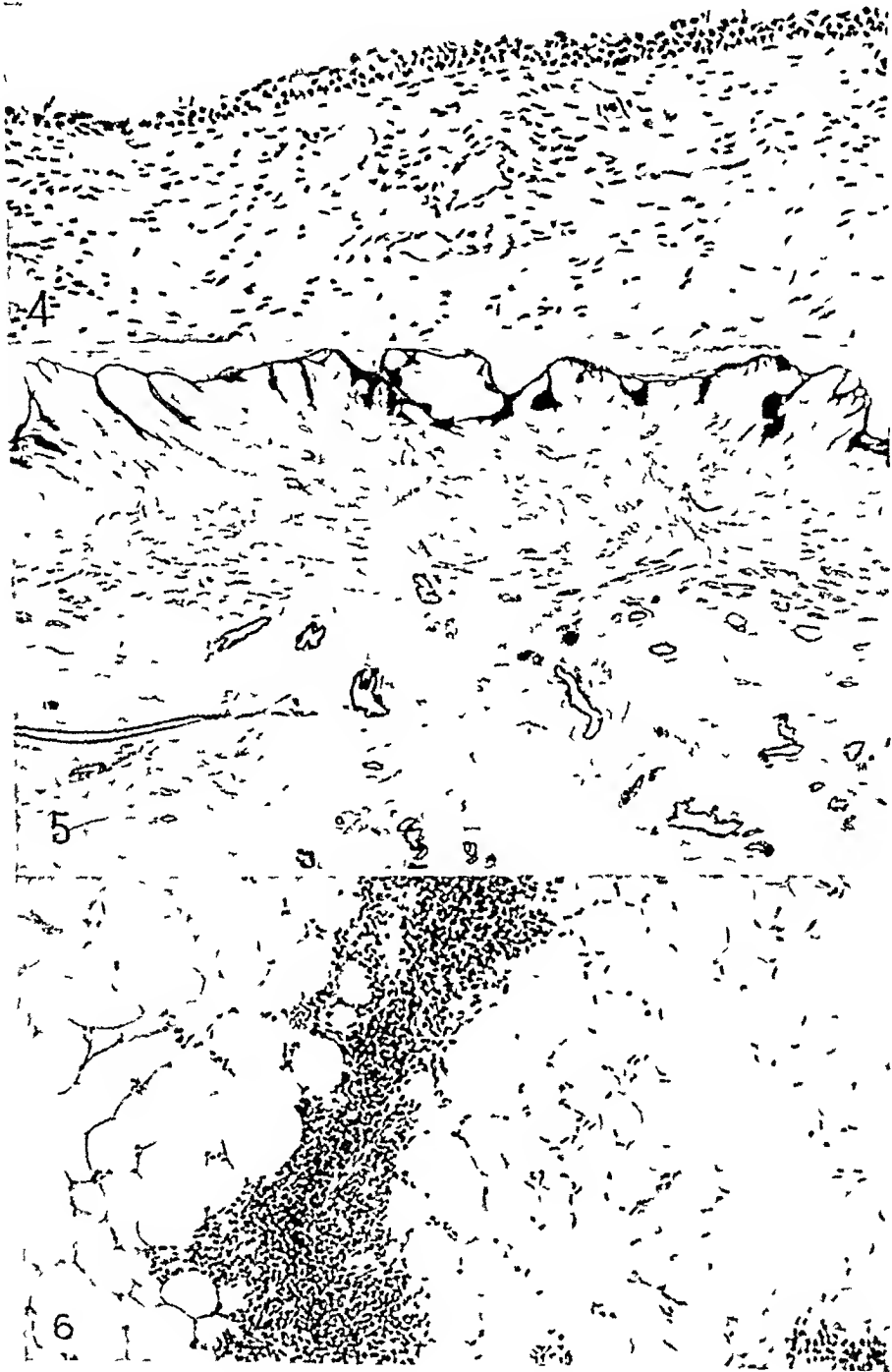


Fig 4—Section of vaginal wall showing thinness of the epithelial lining. Hematoxylin and eosin, $\times 170$.

Fig 5—Section of breast cut through the nipple showing the entire thickness of the organ. Hematoxylin and eosin, $\times 16$.

Fig 6—Thymus showing the marked degree of physiologic involution. Hematoxylin and eosin, $\times 130$.

Pancreas This was normal in size and histologically the exocrine and endocrine tissue appeared normal

Thyroid Gland (12 Gm) The gland was definitely small, but small glands are often found in patients who have had a prolonged wasting illness, so this finding is probably not significant. Microscopically the vesicles were of moderate size, lined by a low cubical epithelium and filled with acidophilic resting colloid. Much brown atrophy pigment was found in the epithelium.

Thymus This showed marked adiposity and floated in water. Microscopically, only a few strands of thymic tissue were found lying in adipose tissue, they consisted of medulla without cortex, and there were only scanty and degenerate Hassall's corpuscles. There was no interlobular fibrosis, and the condition was one of physiologic involution (fig 6).

Pineal Gland This measured 1 by 0.5 by 0.3 cm and showed a central cystic space 0.1 cm in diameter. Microscopically the cells showed small pyknotic nuclei and contained much brown atrophy pigment. A moderate amount of calcification was present.

Pituitary Gland The gland measured 1.6 by 1.2 by 0.8 cm and was within the normal limits of size. Horizontal sections were made at various levels and stained with a modified Mallory's connective tissue stain (McFarlane¹², Mc-Letchie¹³) and a differential count was carried out according to the method of Rasmussen¹⁴. This showed 48 per cent chromophobes, 44 per cent eosinophils and 8 per cent basophils, which is within normal limits¹⁵. There was a moderate amount of basophilic infiltration of the posterior lobe, which showed a considerable amount of brown, prussian blue-negative pigment. The basophils of the anterior lobe were well granulated and showed in some cases a moderate amount of fine vacuolation of a nonspecific type. No hyaline change was found in the cytoplasm of the basophils, and the relative size ratio of the eosinophils and the basophils was maintained. The eosinophils were normally granular and showed no evident abnormality.

Right Kidney Scanty, scarred glomeruli were found, and many calcified casts were seen in the tubules.

Left Kidney This showed almost complete atrophy, only a few unscarred glomeruli being recognized. The tubules were dilated and filled with acidophilic colloid-like material, they showed no calcified casts. There was marked inter-tubular fibrosis of the medulla, which was infiltrated with lymphocytes and plasmacytes.

Whether the lesion of the left kidney was due wholly to previous pyelonephritis or was due in part to hypoplasia is difficult if not impossible to decide, but the presence of plasmacytes, the normality of the artery and the ureter and the fact that the lesions correspond with no known developmental defect are in favor of an acquired lesion.

PATHOLOGIC FEATURES IN PRESENT CASE AND PREVIOUSLY RECORDED CASES OF OVARIAN APLASIA COMPARED

Apart from variations in minor details the findings in all the recorded cases of ovarian aplasia show a close similarity. Detailed descriptions are available

12 McFarlane, D. *Stain Technol* 19 29, 1944

13 McLetchie, N. G. B. *J Endocrinol* 3 323, 1944

14 Rasmussen, A. T. *Am J Path* 5 263, 1929

15 Rasmussen, A. T. *Am J Path* 9 459, 1933

in the literature,¹⁶ and only a summary of the main features will be given under the following headings

Ovarian Tissue—This has been absent from the usual position, and none has been found in ectopic sites, there has been complete absence of sex cells and primordial follicles. Connective tissue thickenings have been described as occurring in the expected position of the ovary in several cases and in some of them pseudoglomerular structures were found. In the present case no such thickenings were recognized but, if present, might well have been obscured by the tuberculous process.

Mullerian Derivatives—In adults the internal genitalia have been underdeveloped and might justifiably be called infantile. In the case of Graber,⁴ that of a 9 week old infant, it is noteworthy that except for the lack of ovaries and slight uterus bicornis arcuatus the genital apparatus was normal in development according to the age.

Wolffian Derivatives—The condition of the structures found in the mesosalpinx and the mesovarium has varied. In the infant described by Graber⁴ the paroophoron was noted as being unusually strongly developed, while in the first case of Pich,² that of an 18 year old girl, a collection of canaliculi resembling underdeveloped epididymis corresponded to the paroophoron. The latter was said by Graber to be rarely found in normal females after 5 years of age.

The epoophoron, which is normally present throughout female life, was said to be absent in the second case of Pich,² while Schurmann⁶ observed that this structure was large enough to form a visible swelling on the serosa, and Rossle and Wallart⁸ noted a marked epoophoron partially cystadenomatous in character. Olivet⁵ did not find any enlargement of this wolffian remnant.

Interstitial cells and tissue resembling the rete ovarii were noted by Rossle and Wallart⁸ and by Pich.² Graber⁴ stated that interstitial cells were absent, while in the reports of other cases cells of this type were not specifically mentioned.

In the present case no paroophoron was found, the tubules of the epoophoron were numerous and in appearance resembled the structure of an epididymis. Interstitial cells were present, embedded in a connective tissue stroma.

Other Endocrine Organs—There have been no constant abnormal findings in the other endocrine organs. The pituitary gland has been variously reported as showing eosinophilia¹⁷ or basophilia,² but in the absence of a differential count arrived at by a standard method¹⁴ little significance can be attached to such observations.¹⁵ In the present case no abnormality was noted in the pituitary gland. The thymus in the case now reported showed marked physiologic involution, and similar findings were reported by Pich.²

Skeletal Development—Retardation of skeletal development has been noted, many of the subjects being below average height and showing late closure of the epiphyses.

In the present case, the height was within the average range, and the epiphyses were closed.

Secondary Sex Characteristics—Except for the variable presence of pubic and axillary hair, the secondary sex characteristics have been absent in adults. Wilkins and Fleischmann¹⁸ pointed out that the presence of pubic and axillary

16 Pich² Rossle and Wallart⁸ Graber⁴

17 Rossle and Wallart⁸ Graber⁴ Olivet⁵ Randerath⁷

18 Wilkins, L., and Fleischmann, W. F. J. Clin. Endocrinol. 4:357, 1944

hair is of importance when one is differentiating aplasia of the ovaries from panhypopituitarism, in which sexual hair is uniformly absent

Coincidental Minor Congenital Abnormalities—While none were present in the case reported here, with the possible exception of the sclerosed left kidney, conditions, such as horseshoe kidney, cervical ribs, fusion of ribs, neurofibromatosis and thinning of the aortic wall have been found

COMMENT

It has recently been shown by Everett¹⁹ that in the mouse the primordial germ cells are originally present in the gut endoderm and later migrate to the genital ridge epithelium. If the genital ridge tissue be transplanted inside the capsule of the kidney of a litter mate of the mother of the donor after the primordial germ cells have reached it, a typical ovary or testis develops but if it is transplanted before they do so, no sex cells will be formed in the genital ridge tissue although the associated genitourinary ducts will develop.

Graber⁴ stated that the primordial sex cells are responsible for the formation of the ovary as a whole, and Wilkins and Fleischmann¹⁸ arrived at a similar conclusion from a study of a series of clinical cases in which biopsies had been performed. By ablation experiments Reagan²⁰ and others showed that in the absence of primordial sex cells connective tissue thickenings may be found in place of the ovaries. Graber⁴ regarded the connective tissue bodies in his case as mesonephric remains because of the presence of regressing pseudoglomerular structures, while Wilkins and Fleischmann¹⁸ explained the position of the connective tissue bodies in their cases as being due to the carrying forward of the primitive genital ridge tissue by the mesentery which forms the broad ligament. It may be noted that in absolute size these bodies are much larger than the original genital ridges, and this may be due to continued growth of this tissue in which the proliferation of an ovarian cortex has not taken place. In the absence of evidence of pathologic destruction of the ovaries either in utero or after birth it seems reasonable to assume that the connective tissue bodies may represent the genital ridge tissue on which the primordial sex cells have failed to induce an ovarian cortex.

That bilateral absence of the ovaries is not produced by bilateral occurrence of the conditions which lead to the more common unilateral defect is shown by the fact that the latter is associated with partial or complete deficiency of the ipsilateral Mullerian duct²¹ and is also frequently associated with renal agenesis on the same side. This seems to suggest that in the cases of unilateral defect there is a failure

19 Everett, N. B. *J. Exper. Zool.* **92** 49, 1943

20 Reagan, F. P. *Anat. Rec.* **11** 489, 1916

21 Shumacker, H. B. *Arch. Surg.* **37** 586, 1938. Varino, G. A., and Beecham, W. D. *Am. J. Obst. & Gynec.* **41** 124, 1941

of normal development of the genital ridge tissue as such, while in the cases of bilateral defect it is only that portion which requires the presence of the primordial germ cells that fails to develop normally.

The more commonly accepted theory of sex determination and differentiation is that the genic balance of the parent germ cell decides the sex and whether an ovary or a testis is to be formed from the undifferentiated gonad. The differentiated gonads then elaborate embryonic sex hormones which guide the development of the genital apparatus. On the other hand, the occurrence of bilateral ovarian aplasia in subjects in which the sex ducts are developed to an infantile stage would appear to support, as far as the female is concerned, the view put forward by Moore,²² that the characters of both the gonad and the sex duct system are genetically determined. In connection with the possible effect of maternal hormones, Wilkins and Fleischmann¹⁸ remarked that this cannot be entirely excluded but that it is difficult to concede the influence of maternal hormones on sex determination unless one grants also that the embryonic testis produces hormones capable of reversing the action of maternal estrogens. That this is not so is evidenced by the first case reported by Altmann²³ of congenital absence of the testes.

It is generally accepted that the presence of normally functioning ovarian tissue is necessary in the female to bring the genitalia to full maturity and to bring about the appearance of the secondary sex characteristics. No departure from this view is necessitated by the findings in these cases of bilateral ovarian aplasia.

Regarding the wolffian remnants, these show great variation in normal subjects, but if one accepts the findings in the few reported cases as a definite though variable hypertrophy, some explanation must be sought. Grabei⁴ expressed the belief that ovarian hormones control the regression of the wolffian remnants and that any enlargement might be explained as being due to an inherent growth tendency of the latter in the absence of ovarian inhibition. Recently Burrows²⁴ has stated that the inhibitory action of estrogen on the wolffian system is slight and inconstant. Ramsay and McCahey²⁵ pointed out that there is a correlation between the amount of rete tissue and associated interstitial cells and the amount of wolffian remains, and that where the former are abundant the latter are also prominent. It is therefore possible that the interstitial cells may produce a secretion which causes growth or inhibits the retrogression of the wolffian remnants. One case has been

22 Moore, C R. *Am Naturalist* **78** 97, 1944

23 Altmann, F. *Virchows Arch f path Anat* **276** 455, 1930

24 Burrows, H. *Biological Actions of the Sex Hormones*, London, Cambridge University Press, 1946

25 Ramsay, A J, and McCahey, J F. *Am J Obst & Gynec* **36** 104, 1938

reported in which enlarged paraurethral ducts were found,² but the finding is of doubtful significance, and there is no real evidence suggesting that the interstitial cells have had any virilizing influence on these subjects. It is possible that when a larger series of cases is available, especially if actual measurements of the wolffian remnants are made, the enlargement may be found to be within the limits of normal variation.

There is no agreement as to the cause of the skeletal immaturity and underdevelopment noted in several of the cases and also in clinical cases of ovarian hypofunction recently reported by Wilkins and Fleischmann¹⁸ and by Albright, Smith and Fraser²⁶. In the case reported here dwarfism is not shown, nor any evidence of skeletal immaturity, and this is considered to favor the view of Wilkins and Fleischmann¹⁸ that there may be a genetic basis for this condition and that the effect of the absence of the ovary, whether it results directly or through lack of stimulation of other endocrine organs, is not at any rate wholly responsible.

SUMMARY

Bilateral absence of the ovaries was observed in an adult female of normal stature. The genitalia, both internal and external, were developed to the infantile stage, there were no secondary sexual characteristics and primary amenorrhea was present. No other congenital anomaly was found.

The fact that this selective congenital defect repeatedly occurs in closely parallel cases, with but minor variations and with no evidence of pathologic destruction, suggests that in these cases the aplasia of the ovaries may be genetically determined.

No specific changes in the other endocrine organs attributable to the absence of the ovaries can be found.

The occurrence of bilateral ovarian aplasia supports the view that the establishment and differentiation of the specific characters of the gonad and duct system which determine the sex of the individual are largely under genetic control.

26 Albright, F., Smith, P. H., and Fraser, R. *Am J M Sc* 204 625, 1942

ECLAMPSIA

Report of a Case in Which There Was Extensive Destruction of the Brain

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AND

EDWIN F HIRSCH, M D

CHICAGO

THE BRAINS of women who have died in an eclamptic attack have been examined rather frequently. In most cases only minor changes have been found. Apparently, few patients surviving eclampsia show late sequelae. Except in the report by Lowenberg and Lossman,¹ there is no description of the brain of a patient who has survived eclampsia for an essential period, after development of severe neuropsychiatric symptoms as a result of the acute attack. Lowenberg and Lossman examined the brain of a woman who had eclampsia and subsequently showed symptoms of severe neurologic disturbance and progressive mental deterioration. She died seven years later. Her brain was atrophic, the cerebral cortex had undergone widespread degeneration, and the centrum semiovale was demyelinated.

Our report describes another brain of this type not only because the condition is rare but because the changes in the brain are those of a patient who survived for only three months after the eclamptic seizures.

REPORT OF CASE

A white girl aged 15 years entered the regular obstetric service at St Luke's Hospital, Chicago, Jan 9, 1945, with the diagnosis of preeclamptic toxemia. When admitted to the prenatal clinic, Sept 15, 1944, she was about five months pregnant. An uneventful course followed until Dec 15, 1944, when marked edema of the ankles was observed, and her blood pressure, which had been 105 mm of mercury systolic and 80 mm diastolic, had risen to 118 mm and 88 mm, respectively. During the following weeks the edema persisted and the blood pressure remained slightly elevated. When admitted on January 9, the patient had a blood pressure of 130 mm systolic and 108 mm diastolic. There was edema of the ankles. The temperature and the pulse rate were not elevated. The pregnancy seemed to be at about eight and a half months. The total nonprotein nitrogen of the blood was 22.6 mg per hundred cubic centimeters. The urine contained 20 mg of albumin per hundred cubic centimeters, but no casts or cells. The blood had 3,440,000 erythrocytes per cubic millimeter and 10 Gm of hemoglobin per hundred cubic centimeters. With rest in bed, the blood pressure remained about 130 mm of

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1 Lowenberg, K, and Lossman, R T. *Am J Path* 19: 697, 1943.

mercury systolic and 110 mm diastolic. On January 12 labor was induced medically with castor oil. During labor the patient had five generalized convulsions. After delivery she had a series of almost continuous convulsions for about forty-five minutes, which were terminated by an intravenous injection of sodium amytal. During these convulsions she became dyspneic and markedly cyanotic. Toward the end of the series the blood pressure dropped to 60 mm systolic and 0 diastolic. Oxygen was given. However, the patient never regained mental consciousness. She lived on for about three months, leading a vegetative form of existence, and was fed by stomach tube. During several weeks following delivery she occasionally had convulsions, which were controlled with sodium amytal. Shortly after the series of convulsions following delivery the spinal fluid was clear, under normal pressure, contained 20 cells per cubic millimeter and had 150 mg of protein per hundred cubic centimeters. No abnormalities were demonstrated by neurologic examinations until January 23, when generalized spasticity was observed, and this persisted until death. Following delivery, her temperature ranged between 101 and 105 F, and on January 18 the urine contained 200 mg of albumin per hundred cubic centimeters, many red blood cells and granular casts. The nonprotein nitrogen of the blood was 32 mg per hundred cubic centimeters. The urine became normal, and several determinations of the plasma proteins of the blood gave values within the normal range. The patient's temperature continued to fluctuate between 101 and 104 F, and the pressure of the spinal fluid gradually increased to 210 mm of water, February 23, but the fluid was clear. A ventriculogram, February 28, demonstrated an atrophic cerebrum and dilated ventricles. The patient continued in a vegetative state and died April 14, three months and two days after the onset of her attacks of convulsions.

Postmortem Examination—The essentials of the anatomic diagnosis following complete postmortem examination were marked encephalomalacia and atrophy of the cortex of the brain, acute purulent tracheitis and bronchitis, atelectasis of the lower lobes of both lungs, cloudy swelling and marked fatty changes of the liver, cloudy swelling of the myocardium and the kidneys, old surgical ventriculographic defect of the left parietal bone and scar of the scalp.

The symmetrically developed body of this young white woman weighed 101 pounds (45.5 Kg) and was 161 cm long. The heart weighed 320 Gm, and there were no significant changes of the valves or of the myocardium. The edematous hyperemic right lung weighed 390 Gm, the left, 350 Gm. Each kidney weighed 160 Gm. The ratio of cortex to medulla and the cortical markings were not unusual. The kidneys showed moderate cloudy swelling. The pancreas had no unusual changes, it weighed 135 Gm. The spleen weighed 230 Gm, and surfaces made by cutting had a dark red pulp with small gray nodules of lymphoid tissue. The liver weighed 1,650 Gm. The lower margins were rounded, the capsule was smooth and the tissues beneath were mottled tan-brown and red-brown. Orange-yellow regions of fatty changes, 1 to 3 mm in diameter, were scattered widely. Surfaces made by cutting had mottlings as mentioned, scattered foci of fatty changes and cloudy swelling.

The brain with the upper half of the dura (approximately 20 Gm) weighed 990 Gm. The spinal fluid was abundant, clear and colorless. The pia-arachnoid at the base of the brain was thin and transparent. The arteries at the base had thin walls. The tissues of the cerebellum, the pons and the brain stem were firm, but those of both cerebral hemispheres, especially of the frontal, temporal and parietal regions, were soft, the convolutions were atrophic and flattened and the sulci narrowed. In the posterior portions of the parietal and the occipital lobes

the convolutions were rounded and the sulci wider. There were no unusual changes in the accessory sinuses of the cranium or in those of the dura. The examination of the cervical structures and of the right knee revealed no significant changes.

After formaldehyde fixation there were no abnormal adhesions between the dura and the brain. A small subdural hematoma, 5 by 5 cm., was present in the region of the wound resulting from the needle puncture made in the posterior portion of the left parietal lobe in taking a ventriculogram. The configuration of the brain has been mentioned. The cerebral hemispheres were soft. In coronal sections of the cerebrum, the cortex was narrowed and generally separated from the centrum ovale by a narrow layer of loose tissue parallel to the gyral surface. The white matter was pale and friable, especially the poles of the temporal lobes seemed to be softened. The basal ganglions were normal, and the lateral ventricles were moderately dilated. The cerebellum, the pons and the brain stem showed no gross changes.

Histologic Examination of the Brain—Tissues from many regions of the brain were embedded in celloidin (a concentrated preparation of pyroxylin), paraffin or gelatin. Sections were stained with toluidine blue, by Van Gieson's method, and for myelin sheaths, for neurofibrils, for mesenchymal structures with tannin silver, and for lipids.

The subdural hematoma was partially organized. In the fibroplastic tissues many phagocytes contained granular blood pigment. The pia-arachnoid generally was thickened by fibrous and fibroplastic tissues. The veins were dilated, and occasionally there were extravasations. A coronal section of the cerebral hemispheres, stained for myelin sheaths (fig 1A), disclosed marked changes of the tissues. The white matter of the parietal lobe and of the second and third temporal gyri stained poorly and was much lighter than that of the darkly stained first temporal gyrus. The cortex of the temporal and parietal lobes was narrow, and the edge bordering the white matter was more sharply defined than usual. The cortex in the temporal lobe had separated from the white matter in many places. The frontal gyri had narrow, long, stretched focal regions of demyelination, which extended parallel to the gyral margin. These were in the lower part of the cortex, or at the border between the cortex and the white matter, and were sharply outlined (fig 1B). Marked and extensive destruction of the cortex was demonstrated by sections stained with toluidine blue. This was especially marked in the anterior portions of the cerebral hemispheres but occurred also in the occipital region, where in many places the usual tissue structure remained. The tissue changes in the cortex can be arranged into two main groups. In each there was marked loss of the nerve cells and fibers. The difference between them was in the subsequent reaction. In one there was an accumulation of Hortega and gitter cells, associated with a marked growth of mesenchyma, that is, a softening with organization, in the other there was proliferation only of the glia, which had replaced the nervous parenchyma, without gitter cells or an increase of mesenchyma. These forms of reaction were not separated strictly but occurred together in many foci (figs 2A and B, 3A and B, 4A). They were found in many places in the deeper portions of the cortex or at the border between the cortex and the white matter. They seemed to account for the separation of the cortex and the centrum semiovale. Such softenings, however, occurred also in the outer layers of the cortex or had destroyed the entire thickness of the cortical tissues. They were small and seemed not to correspond to the distribution of an artery. In a few places with mesenchymal proliferation the cortex had undergone coagulation necrosis, the tissues being reduced to a homogeneous mass without



Fig 1—*A*, coronal section of left cerebral hemisphere. Myelin sheath staining. Note demyelination of the centrum semiovale in the frontal lobe, as well as in the second and the third temporal gyrus, and the destruction and separation of the cortex and the circumscribed foci of necrosis in the first and the second frontal gyrus. *B*, one of the foci of necrosis. Note the sharp border, also the engorged blood vessels and hemorrhage in the center.



Fig 2—*A*, area of softening of the cortex exhibiting many gitter cells. Toluidine blue staining. Medium power enlargement.

B, long, stretched, narrow focus of softening in the cortex. Glia proliferation is seen in the upper layers, and newly formed vessels in laminae III and IV. Toluidine blue staining. Medium power enlargement.

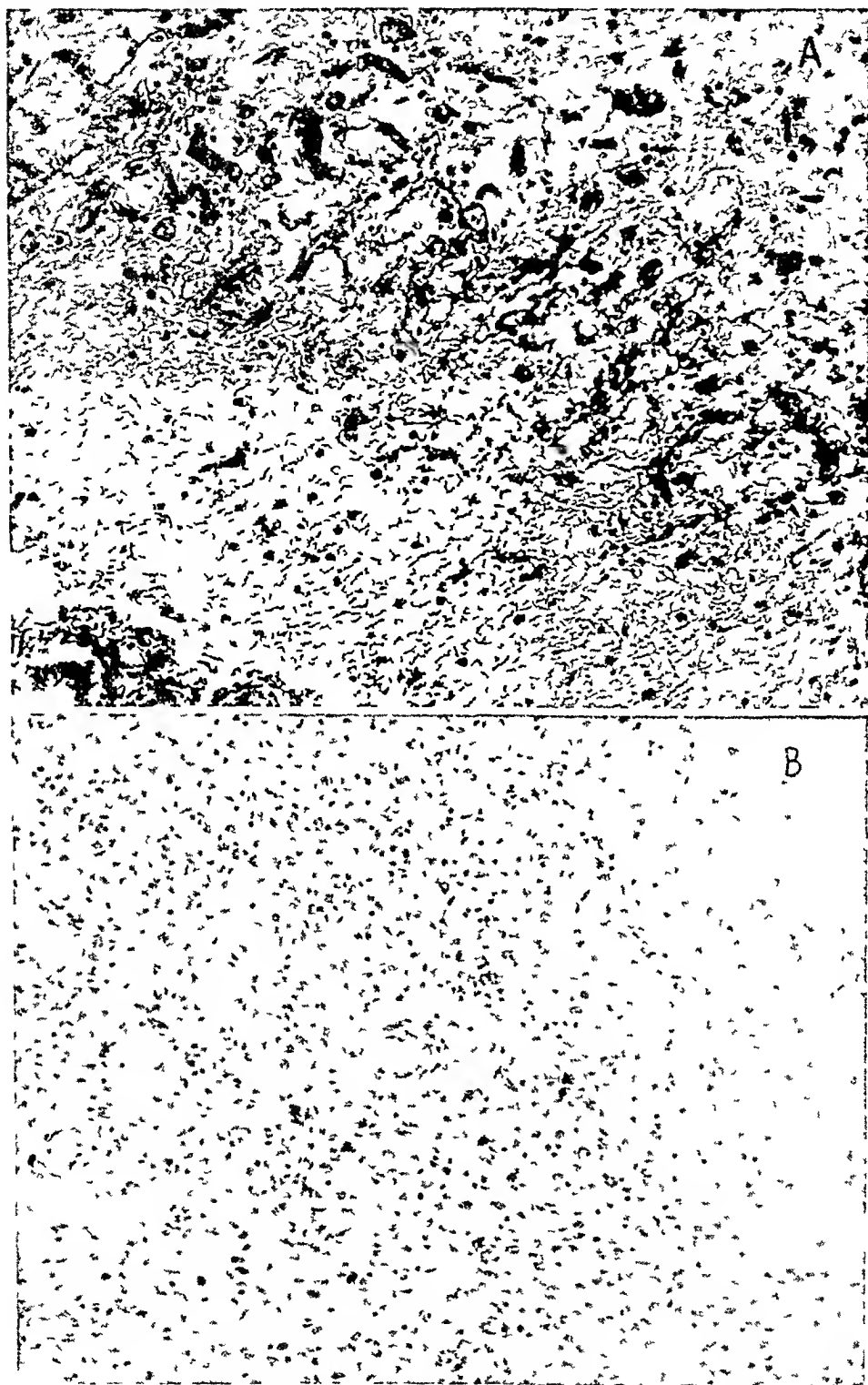


Fig 3—*A*, the same focus as in figure 2 *B*. Tannin silver staining to demonstrate the proliferation of the mesenchyma.

B, marked destruction of the cortex. Glia proliferation without reaction of the mesenchyma. Toluidine blue staining. Medium power enlargement.



Fig 4—*A*, cerebellum. Focus of Hortega cells in the molecular layer. Toluidine blue staining. Medium power enlargement.

B, venule and capillary from the cortex. Both are engorged with tightly packed red blood cells. The venule contains a small thrombus. Van Gieson's staining. Medium power enlargement.

cells or nuclei. The capillaries and the veins of the cortex were markedly dilated, the walls being thin and formed by a single layer of endothelial cells containing fine droplets of lipid and of a few connective tissue fibrils. There were a few small platelet thrombi (fig 4B). The perivascular spaces were dilated, and occasionally small hemorrhages were observed. In the region of demyelination of the white matter the myelin sheaths had marked retrogressive changes. They were swollen or reduced to small particles and granules. The veins of the centrum ovale were dilated and had wide Virchow-Robin spaces. Sections stained with toluidine blue demonstrated proliferation of the Hortega cells and astrocytes. There were a few foci of softening in the outer capsule and around the anterior horn of the lateral ventricle.

The basal ganglions, the thalamus and the hypothalamus, the nucleus ruber, the substantia nigra and the medulla oblongata had no changes. The Purkinje cells had disappeared in large portions of the cerebellum, without reaction of the glia. Silver-stained preparations disclosed empty baskets. The cortex had small foci of Hortega cells (fig 4A). The nerve cells in the dentate nucleus were reduced markedly in number, and in the white matter there was marked proliferation of the microglia.

Histologic Examination of Other Viscera—Changes were observed in the liver, the kidneys and the lungs. The liver had foci of fatty changes of the hepatic cells, but in these regions the lobular structure of the tissues was present. Elsewhere the hepatic cells and cords showed no significant changes. The capillary tufts of the renal glomeruli were cellular, had hyaline thickenings and some also a few polymorphonuclear leukocytes. The lungs had marked catarrhal bronchitis and foci of bronchopneumonia.

COMMENT

The pathologic observations in the brain of this patient can be summarized briefly as those of a destructive process of the cortex which three months after the onset of the convulsive seizures of eclampsia was in a state of more or less advanced organization and scarification. The centrum semiovale had demyelination. The cerebellum was affected also but to a much less degree than the cerebral hemispheres. These changes are identical basically with those described by Lowenberg and Lossman¹ in their report on atrophy of the brain following eclampsia. The differences between their case and ours are essentially those dependent on the duration of the process. Lowenberg and Lossman examined a brain seven years after the eclampsia occurred, our patient died three months after the initial convulsions. Accordingly, Lowenberg and Lossman saw the final outcome of a process which we observed in a rather early, active stage. Neither Lowenberg and Lossman nor we found another report of eclampsia with similar, and as extensive, changes of the brain.

Minor injury of the central nervous system and even major damage in the form of hemorrhages have been described more frequently. Levant and Portes² held that hemorrhages occur in the central nervous

2 Levant and Portes, L. *Gynéc et obst* 7 332, 1923

system less frequently than is commonly assumed, extravasates are present in only 27 per cent of all cases of eclampsia. They examined 46 brains with extravasates. They observed hemorrhages in the meninges in 20, in the cerebrum and the meninges in 10 and in the cerebrum in 11. Welch³ observed a large hemorrhage in the striatum. Wegelin⁴ reported the same. Von Braunmühl⁵ made a careful study of the brains of 2 women who died in the convulsions of eclampsia. In both he found diffuse damage of the nervous parenchyma and also foci of necrobiosis connected evidently with disturbances of the blood supply. He expressed the belief that vasospasms rather than venous stasis had caused the damage. Sioli⁶ found the same changes, that is, diffuse degeneration and focal lesions. He maintained that stasis and thrombosis of small vessels are responsible and added that in all brains examined fatty degeneration of endothelial and other cells of the vascular walls was present.

Heynemann⁷ and others reported identical findings. Weiman,⁸ in a resumé of the literature up to 1930, stated that in eclampsia the brain may have the signs of disturbances of the circulation with all the changes resulting from it. Diamond⁹ described the brains of 5 patients, all of whom died in coma soon after the onset of eclampsia. In all, pathologic changes were present in the central nervous system. They were general or diffuse as well as focal. The diffuse pathologic changes were more marked than the focal. There were edema, degeneration of ganglion cells and reaction of the glia and of the microglia and of the meninges. Two brains disclosed inflammation, considered by the author as a form of aseptic meningitis. The focal lesions, such as small softening, minute and massive hemorrhages and accumulations of Hortega cells, occurred less frequently than the diffuse damage.

These focal changes may represent on a small scale lesions which are identical with those we found in the form of an extensive process.

In general, the opinion of all authors seems to be that these disturbances of circulation are functional and without actual damage of the vessels. However, Winkelman¹⁰ in a case of chorea in pregnancy found marked changes of the vessels. The vessels were narrow and

3 Welch, J. E. *Bull. Lyng-in Hosp.*, New York **6** 12, 1909.

4 Wegelin. *Berl. klin. Wchnschr.* **44** 2094, 1909.

5 von Braunmühl, A. *Ztschr. f. d. ges. Neurol. u. Psychiat.* **117** 698, 1928.

6 Sioli, F., in Hinselmann, H., and others. *Die Eklampsie*, Bonn, F. Cohen, 1924, p. 559.

7 Heynemann, T., in von Lichtenberg, A., Voelcker, F., and Wildbolz, H. *Handbuch der Urologie*, Berlin, Julius Springer, 1928, vol. 3, pt. 1, pp. 599-652.

8 Weiman, W., in Bumke, O. *Handbuch der Geisteskrankheiten*, Berlin, Julius Springer, 1930, vol. 7.

9 Diamond, I. B. *Arch. Neurol. & Psychiat.* **35** 1320, 1936.

10 Winkelman, N. W. *Ztschr. f. d. ges. Neurol. u. Psychiat.* **102** 56, 1926.

had proliferation of the endothelial and adventitial elements. His case differed from those of the aforementioned authors insofar as his patient was sick with chorea for six months during pregnancy, in contrast with the eclamptic patients who died in acute convulsions after a short illness. Lowenberg and Lossman,¹ in discussing the genesis of the disease of their patient, assume that the initial process was necrosis of the nervous parenchyma and that it must have been caused either by functional disturbances of the circulation of the blood or by toxic influences. They decided that toxic factors acting directly on the parenchyma were responsible for the extensive damage, as a vascular factor may cause diffuse necrosis of the cortex but not of the white matter.

The histopathologic changes observed in the brain of our patient point to a vascular genesis rather than to primary degeneration of the nervous parenchyma. The small softenings found in the cortex and also in the white matter are, according to experience, definitely connected with some disturbance of the blood supply. The same reparative proliferation of the glia without involvement of the mesenchyma which was present in the cortex is a common finding in the sclerosis of the cornu ammonis in epilepsy. This sclerosis according to Spielmeyer¹¹ and others is due to a vascular factor. Furthermore, the enormous dilatation of capillaries and small veins is a striking feature in our case. It seems justified to assume that this vasodilation has played an important role in the genesis of the widespread focal lesions. Recently Scheinker¹² has described as vasoparalysis and vasothrombosis of the central nervous system two related conditions from which extensive and marked damage may result. The damage is vascular in origin; it is not bound to regions supplied by a small or a large artery but to those supplied and drained by capillaries and small veins. The morphologic criteria for vasoparalysis are given by Scheinker as (1) maximal distention and engorgement of the smaller veins and capillaries, (2) signs of stasis consisting in hemolysis of red blood cells, (3) degenerative changes or complete necrosis of vessel walls with increased permeability for serous fluid and red blood cells and (4) distention of the perivascular spaces, which usually contain extravasates. In venous thrombosis the wall of the vessel may appear healthy and have a well preserved endothelial lining. Adherence of the blood clot, composed of red cells, platelets and fibrin, cannot be expected, although the mere presence of clots or an amorphous mass of agglutinated red blood cells with a large accumulation of platelets and large curved strands of fibrin which fills completely the lumen of an enormously distended small venule or capillary is sufficient evidence of venous thrombosis. If these

11 Spielmeyer, W. *Arch Neurol & Psychiat* **23** 869, 1930

12 Scheinker, I. M. *Arch Neurol & Psychiat* **52** 43, 1944 **53** 171, 1945

changes are associated with secondary lesions of the adjacent nerve tissue that are typical of vascular occlusion and perivascular in distribution, there is little doubt that the venous occlusion ought to be regarded as thrombotic

Our observations match the essential features of Scheinker's description. Some of the illustrations in Scheinker's report could be as well photographs taken from the brain described here. Therefore, we conclude that a vascular factor, namely, vasoparalysis, as well as venous thrombosis, is responsible for most of the lesions described. However, it is possible that a toxic factor, such as anoxemia, has caused the rather diffuse demyelination of the centrum semiovale, which in our case is less impressive than that observed in the case of Lowenberg and Lossman.

Only a brief comment is necessary concerning the relation of the clinical symptoms and the pathologic alterations in our case. The destruction of brain is extensive and intensive enough to explain the condition of decerebrate rigidity.

Finally, the question arises whether cases like that of Lowenberg and Lossman and ours are as rare as would appear from the reports in the literature. Psychotic episodes in women suffering from eclampsia are assumed to last only a short time and to terminate in complete recovery (Sioli¹³). Few reports indicate more severe and longer persisting damage. Sioli referred to 2 cases published by Westphal in 1908 and Wichura in 1912, respectively. The first author observed a psychosis after eclampsia with symptoms of aphasia, agraphia and apraxia, the patient seemed well after one year. Wichura observed a woman who two years after the onset of an eclamptic psychosis had severe organic neuropsychiatric disturbances involving memory, gnosis and praxis. However, there have been neither systematic neuropsychiatric examinations of patients who have survived eclampsia for a long time nor studies of the brains of such patients. Probably damage of the central nervous system occurs more frequently than has been reported. If destruction of nervous parenchyma is less extensive and intensive than in our case and the one described by Lowenberg and Lossman, clinical symptoms may be inconspicuous and overlooked.

SUMMARY

Eclamptic convulsions in a young woman were followed by a vegetative existence until death three months later. The brain had extensive regions of destruction in various stages of organization. The destruction was caused by vascular disturbances, especially by paralysis and thrombosis of venules and capillaries. There is only one other description of this condition in the literature.

13 Sioli, F., in Hinselmann, H., and others. Die Eklampsie, Bonn, F. Cohen, 1924.

TEMPORAL ARTERITIS

Report of a Case and a Comparison with Respect to Periarteritis Nodosa

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IN 1932, Horton, Magath and Brown¹ reported 2 cases of arteritis of the temporal vessels associated with fever, anorexia, malaise, anemia, loss of weight and pain in areas along the temporal arteries and over the scalp. The clinical course, ending with recovery, and the pathologic features observed in biopsy specimens did not suggest any of the known forms of arteritis, and the authors were inclined to believe that their cases represented a new clinical and pathologic entity.

Subsequent reports have added greatly to the clinical picture of temporal arteritis or, to speak more correctly, have revealed the great diversity of symptoms possible with this condition. Pain occurring in teeth,¹ ear,² jaw² and occiput³ suggested involvement of other branches of the external carotid artery. Relatively frequent ocular symptoms,⁴ occasional symptoms suggesting cerebral damage,⁵ and pain of the extremities^{4a} added to the possibility that this condition may be associated with widespread involvement of the arterial tree and not necessarily with local involvement of the temporal arteries only.

Gilmour⁶ reported the necropsy observation in 4 cases resembling those of temporal arteritis in their symptoms. His observations revealed widespread arteritis with involvement of the aorta, the internal and the external carotid artery and the subclavian, innominate, cerebral and

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1 Horton, B T, Magath, T B, and Brown, G E. Proc Staff Meet, Mayo Clin **7** 700, 1932

2 Horton, B T, and Magath, T B. Proc Staff Meet, Mayo Clin **12** 548, 1937

3 Bowers, J M. Arch Int Med **66** 384, 1940

4 (a) Jennings, G H. Lancet **1** 424, 1938. (b) Scott, T, and Maxwell, E S. Internat Clin **2** 220, 1941. (c) Johnson, R H, Harley, R D, and Horton, B T. Am J Ophth **26** 147, 1943. (d) Shannon, E W, and Solomon, J. J A M A **127** 647, 1945. (e) Horton and Magath²

5 Bain, C W C. Lancet **1** 517, 1938. Sprague, P H, and MacKenzie, W C. Canad M A J **43** 562, 1940. Schaefer, C L, and Sanders, C E. Am Heart J **24** 410, 1942

6 Gilmour, J R. J Path & Bact **53** 263, 1941

common iliac arteries. He considered the pathologic condition a form of chronic arteritis characterized by multinucleated giant cells—a pathologic picture similar to that seen in temporal arteritis. In the aorta the lesions were widespread, in the carotid arteries the distribution of the lesions was focal, bilateral and symmetric. He reported no involvement of the temporal arteries. Final proof that temporal arteritis is but a local manifestation of widespread arteritis is found in the following reports on the necropsy findings of typical cases. Sproul⁷ observed profound changes in the temporal, carotid, innominate, subclavian, pulmonary, celiac, mesenteric, renal and iliac arteries and in the aorta. Chasnoff and Vorzimer⁸ reported diffuse involvement of the arterial tree. Cooke and his associates⁹ described characteristic pathologic changes in 2 cases, with involvement of the aorta and the temporal, radial, subclavian, femoral, coronary, renal, retinal, celiac and mesenteric arteries.

Temporal arteritis is relatively rare, reports of approximately 40 cases being found in the literature. The rarity of this condition and an opportunity to compare it pathologically with periarteritis nodosa as seen in the material from 7 necropsies prompts us to report our case.

REPORT OF CASE

A 65 year old white laborer entered the Rochester General Hospital in August 1945, complaining of severe headache and pain over both temporal regions. Eight weeks prior to his admission pain had developed in both thighs. This pain was aggravated by motion and was accompanied by fatigue, malaise and anorexia. Soon afterward he noticed a dull ache in his left shoulder. Two weeks after the onset of his symptoms he was unable to continue on his job and went to bed. Four weeks before admission extreme bitemporal pain and frontal headache developed. Both anterior temporal arteries became swollen and extremely tender, and superficial burning pain was present in the surrounding areas of skin. The pain and the headache were constant but seemed worse at night. Analgesics afforded little relief. The patient stated that he had lost 20 pounds (9 Kg) in weight during the eight weeks prior to hospitalization.

Before this illness his general health had been excellent. Nocturia had occurred three to four times in the past three years, and there was a vague history of "rheumatism" in the left shoulder.

On admission he appeared pale and gave the impression of a chronically ill patient. The anterior temporal arteries were prominent, enlarged and tortuous (fig 1). On palpation, both vessels were found to be thickened, tender and faintly pulsating. There were red streaks in the skin about the walls of the left temporal artery. Examination of the eyegrounds revealed no significant pathologic change. The radial and pedal arteries were slightly thickened, their pulsations were forceful and regular at a rate of 80 per minute. His few remaining teeth

⁷ Sproul, E. E. *New York State J. Med.* **42** 345, 1942.

⁸ Chasnoff, J., and Vorzimer, J. J. *Ann. Int. Med.* **20** 327, 1944.

⁹ Cooke, W. T., Cloake, P. C. P., Govan, A. D. T., and Colbeck, J. C. *Quart. J. Med.* **15** 47, 1946.

were dirty and carious, with periapical disease and pyorrhea. There was no detectable cardiac enlargement, and the cardiac sounds were faint. The prostate was enlarged to twice normal size and firm. Passive flexion of either thigh produced pain in the groin. The blood pressure was 120 systolic and 80 diastolic. The rectal temperature was 99 F.

The red cell count was 3,590,000 and the white cell count 8,700, with 71 per cent segmented neutrophils, 2 per cent eosinophils, 3 per cent monocytes and 24 per cent lymphocytes. The hemoglobin content was 10.2 Gm. The corrected erythrocyte sedimentation rate was 29 mm in one hour (Wintrobe), with a hematocrit reading of 32 per cent. Chemical examination of the blood gave the



Fig 1—Prominent, tortuous, temporal artery of a patient with bilateral temporal arteritis

following values: blood nonprotein nitrogen 28 mg, blood glucose 79 mg, blood phosphorus 4.6 mg, serum alkaline phosphatase 495 units, total serum protein 6.8 mg, serum albumin 3.3 mg and serum globulin 3.5 mg per hundred cubic centimeters. Gastric analysis revealed no free hydrochloric acid but a good response following administration of histamine. Culture of material from the throat revealed 80 per cent nonhemolytic streptococci and 20 per cent staphylococci (*Staph. albus*). Two blood cultures were reported sterile after eight days. Agglutination tests for *Bacillus typhosus*, *Salmonella paratyphi*, *Salmonella schottmuller* and *Brucella abortus* were reported negative. The Wassermann test of the blood was negative. Urinalysis revealed no significant abnormality.

Roentgenograms revealed a well healed small fibrotic lesion in the apex of the left lung, and no abnormality of the bones of the pelvis or of the upper parts of the femurs. The electrocardiogram showed a normal pattern.

During the first four hospital days the rectal temperature ranged from 99 to 101 F and thereafter was normal. On the second hospital day the temporal pain subsided rather abruptly, and the pain in the groins subsided slowly over the next



Fig 2—Low power photomicrograph of a temporal artery showing diffuse involvement of all coats with almost complete obliteration of the lumen.

fourteen days. The constitutional symptoms improved gradually, and a gain of 5 pounds (2.5 Kg) was noted when the patient was discharged on the eighteenth hospital day. Biopsy specimens, each about 2 cm in length, were excised from both anterior temporal arteries on the fourth hospital day.

In April 1946, eight months after hospitalization, the patient returned for a follow-up examination. He had resumed his work as a laborer two months after

being discharged from the hospital and was essentially asymptomatic. He had regained his former weight and strength. The arteries in the temporal regions were no longer visible or palpable, however, pulsations were felt in both main superficial temporal arteries behind the respective temporomandibular articulations. Several laboratory tests were repeated at this time. The blood count showed no anemia. The erythrocyte sedimentation rate was 33 mm in one hour.

Histologic Examination—Numerous sections of the arterial specimens through all levels revealed essentially similar changes. All layers of the vessel wall were thickened and infiltrated in varying degrees of intensity, predominantly by lymphoid cells (fig 2). The intima revealed a proliferation of loose connective tissue that completely obliterated the lumen except for a few small recanalized lumens. The connective tissue appeared to be edematous and was infiltrated by small numbers of lymphocytes and plasma cells. The internal elastic membrane was reduplicated, fragmented and completely destroyed in a number of places (fig 3). The media was edematous and infiltrated by large numbers of lymphocytes, plasma cells



Fig 3—High power photomicrograph of tissue prepared with a stain for elastic tissue to show the reduplication, fragmentation and degenerative changes in the internal elastic membrane

and occasional neutrophil leukocytes. Small focal areas of necrosis were numerous throughout the media, but they showed a definite tendency to be arranged in contact with the internal elastic membrane. About these necrotic areas epithelioid cells were arranged in palisade formation. Moderate numbers of multinucleated giant cells were also present in these granulomatous lesions (fig 4A). There was a dense infiltrate of lymphoid cells in the adventitia, particularly in the form of collars about the vasa vasorum.

COMMENT

As in most cases of temporal arteritis reported, general malaise, weakness, anorexia and loss of weight were outstanding symptoms in this case. Our patient had the appearance of one debilitated by a long-standing illness. With the recently acquired knowledge that this con-

dition is polyarteritis we find it reasonable to associate these symptoms with the involvement of arteries other than the temporal This sup-

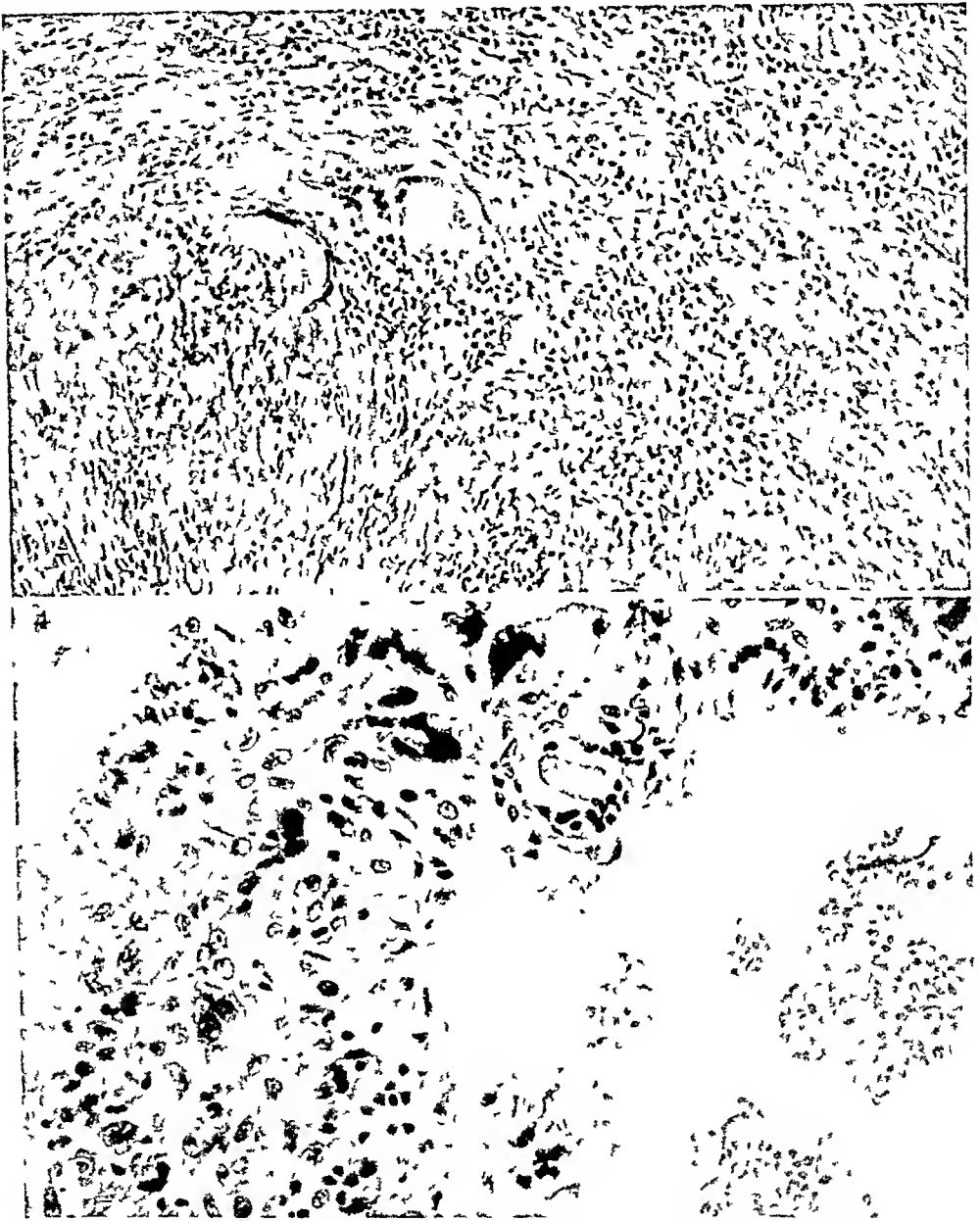


Fig 4—*A*, high power photomicrograph of tissue stained by the hematoxylin-eosin method, showing areas of necrosis, epithelioid cells in palisade formation, multinucleated giant cells and diffuse cellular infiltration of the media

B, high power photomicrograph of a section of a coronary artery of a patient with periarteritis nodosa, showing many multinucleated giant cells, hematoxylin and eosin stain

position is strengthened by the fact that in our case the initial symptom was pain in the extremities. With the appearance of involvement of the temporal arteries, severe headache and extreme bitemporal pain became

the prominent symptoms. The succession of symptoms suggests progressive involvement of different arteries.

The moderate hypochromic anemia, the slight leukocytosis, the increased sedimentation rate and the moderately elevated temperature are frequent findings that have suggested to many an infectious basis for temporal arteritis. As in all other attempts, our bacteriologic and serologic studies revealed no bacterial agent.

It is to be noted that in our case the bitemporal pain had subsided before the biopsy specimens were taken.

A COMPARISON OF TEMPORAL ARTERITIS AND PERIARTERITIS NODOSA

In the following discussion we proceed on the assumption that it is valid to include with the typical cases of temporal arteritis those cases in which necropsy revealed involvement of the aorta and its large branches, with or without involvement of the temporal arteries.

Horton and his associates¹⁰ described temporal arteritis as a form of periarteritis and arteritis characterized by granuloma-like lesions of the adventitia, by round cell infiltration about the vasa vasorum of the adventitia and by similar but less extensive infiltration of the media. They found hemorrhages in the media and marked intimal thickening in many places. They expressed the view that the lesion probably began as periarteritis in a small segment of the affected artery. This pathologic picture, they said, differs from that of periarteritis nodosa in that the inflammatory cells in the latter occur in the form of nodules in the wall of the vessel, and these nodules are made up of collections of mononuclear, polymorphonuclear and eosinophil leukocytes, but they asserted giant cells have not been observed in periarteritis nodosa. Another difference, they pointed out, is that in periarteritis nodosa necrosis of the media frequently leads to formation of aneurysms. Aneurysm formation has not been reported in unequivocal cases of temporal arteritis. We feel that the differentiation of temporal arteritis from periarteritis nodosa on the basis of cellular response in the two conditions is impossible. A diffuse infiltration of the vessel wall in which polymorphonuclear and eosinophil leukocytes participated is mentioned in a number of reports on temporal arteritis.¹¹ Furthermore, the presence of multinucleated giant cells in periarteritis nodosa has been reported in the literature. A brief search revealed 2 cases.¹² Undoubtedly, a more thoroughgoing search would

10 Horton, B. T., Magath, T. B., and Brown, G. E. *Arch. Int. Med.* **53**, 400, 1934.

11 Hoyt, L. H., Perera, G. A., and Karwar, A. J. *New England J. Med.* **225**, 283, 1941. Kilbourne, E. D., and Wolff, H. G. *Ann. Int. Med.* **24**, 1, 1946. Bowers³.

12 Krahulik, L., Rosenthal, M., and Loughlin, E. H. *Am. J. M. Sc.* **190**, 308, 1935. Haining, R. B., and Kimball, T. S. *Am. J. Path.* **10**, 349, 1934.

reveal a higher incidence. Among our 7 cases of periarteritis nodosa 2 showed the presence of giant cells. The section reproduced in figure 4 B was cut through a coronary artery of a 17 year old boy in a typical case, with extensive involvement of most of the visceral arteries.

Cooke and his associates stated that in some specimens it was impossible to make a distinction between temporal arteritis, periarteritis nodosa and thromboangitis obliterans, however, they did make certain generalizations. With regard to periarteritis nodosa they stated

The reaction is of acute inflammatory character, and the necrosis of the vessel wall tends to be of a suppurative nature. In temporal arteritis necrosis is found apart from inflammation, and when inflammation is found it is rarely more than subacute. This inflammatory reaction appears to spread axially, as opposed to the focal nature of periarteritis nodosa.

We find no validity in this generalization. The lesion in periarteritis nodosa is never suppurative. Pyogenic bacteria are not found, and the cellular infiltrate does not form pus. The term "suppurative" evidently is used to describe the dense leukocytic infiltration often seen in periarteritis nodosa. As already mentioned, a similar infiltration of the entire wall of the vessel has been observed a number of times in temporal arteritis. Characteristically, periarteritis nodosa presents a great variety of histologic pictures. This variety is determined by the fact that the pathologic process goes through both destructive and reparative phases, and furthermore certain aspects of the histologic picture are the resultant of the rate of destruction and repair. Initially the lesion in periarteritis nodosa is often characterized by medial necrosis associated with little or no inflammatory exudate. This phase is followed by an acute inflammatory reaction, often involving the entire wall of the artery, with polymorphonuclear neutrophils, sometimes many eosinophils, lymphocytes, plasma cells and occasionally giant cells. Reparative phases follow, with proliferation of fibroblasts and formation of granulation tissue. In occasional cases a healed phase with scar tissue may be found. The pathologic pictures of temporal arteritis already described, strongly point to similar destructive and reparative phases. Obviously it is invalid to choose one phase in a pathologic process and label it as the only one representative of that condition. The picture of necrosis with little or no inflammatory reaction described by Cooke and his associates is no more and no less typical for temporal arteritis than the dense inflammatory reaction described in other reports, the same is true of periarteritis nodosa. We are convinced that every histologic picture described in temporal arteritis so far can be found in periarteritis nodosa.

The axial spread in temporal arteritis as opposed to the focal nature of periarteritis nodosa requires little comment. As is well known, the focal or nodose character is not always present. In one of our cases the gross diagnosis was missed at necropsy despite the clinical diagnosis.

of periarteritis nodosa. The microscopic sections revealed diffuse involvement with no tendency toward nodule formation. Also it is of interest to note that Gilmour described the distribution of lesions of the carotid arteries in his group of cases as being focal.

There are important differences between temporal arteritis and periarteritis nodosa. Whether these differences are due to different causative factors cannot be answered at present. However, the differences are such as to have value in diagnosis and prognosis and for practical reasons they justify considering the two as separate entities. The differences are found in the distribution of the lesions, in the age and sex groups involved and in the mortality rates.

Periarteritis nodosa involves the visceral arteries and only less commonly a few of the medium-sized and smaller peripheral arteries. We could find no reference in the literature on periarteritis nodosa, available to us, of involvement of the aorta or its primary peripheral branches or both. From the few reports of necropsies it is apparent that the visceral arteries may be involved in temporal arteritis but that the predominant involvement is found in the aorta and in its large branches, such as the innominate, carotid, subclavian and iliac arteries and others.

Temporal arteritis is observed twice as frequently in women, however, no great significance should be placed on this sex difference in so small a group. The age group is striking, for in no unequivocal case, reported has the patient been below the age of 55. In striking contrast, periarteritis nodosa occurs at all ages and is most frequent in young adult males.

That the different distribution of lesions determines the different prognosis of each condition can only be a surmise, but the fact remains that periarteritis nodosa is usually a fatal condition, reports of recovery being extremely rare. Although the generally assumed favorable prognosis of temporal arteritis is no longer tenable, a large proportion of the patients do recover.

The term "periarteritis nodosa" and "temporal arteritis" are inadequate in that they do not describe the essential elements of the conditions to which they are applied and should distinguish as entities. Periarteritis nodosa is rarely only periarteritis, the nodose character, although commonly present, is not essential in the delimitation of this from other forms of arteritis, and not infrequently it is entirely absent. Similarly, temporal arteritis is not limited to the temporal arteries, and what is even more disconcerting, the temporal arteries may not be involved. At present the nomenclature, of necessity, must remain on a descriptive level, and herein lies the difficulty. Logically periarteritis nodosa might be renamed "panarteritis predominantly of the visceral arteries" and temporal arteritis "panarteritis predominantly of the aorta and its peripheral branches." Obviously such terms are awkward and not acceptable.

Furthermore, the original terms have become fixed in the minds of physicians and are associated with definite clinical pictures. We strongly suspect that despite their shortcomings the original terms will be maintained.

CONCLUSIONS

Both periarteritis nodosa and temporal arteritis go through destructive and reparative phases, histologic pictures essentially similar to those described in temporal arteritis can be seen in periarteritis nodosa.

The distribution of lesions is different in the two conditions. Essentially, periarteritis nodosa is panarteritis predominantly of the visceral arteries, and temporal arteritis is panarteritis predominantly of the aorta and its peripheral branches.

The sex and age groups involved and the mortality rates of the two conditions are different.

LESIONS OF RETICULOENDOTHELIAL CELLS IN ANAPHYLACTIC SHOCK

P BUENO

SAO PAULO, BRAZIL

A FOREIGN PROTEIN parenterally introduced into an organism previously treated with the same substance may give way to general manifestations of hypersensitivity or local inflammatory changes similar to those observed in the Arthus phenomenon

Though the occurrence of a lesion of an individual cell has been admitted for a better understanding of the anaphylactic reaction, no morphologic substratum has been evidenced. In fact, a change in the structure of a cell consequent to a specific hypersensitivity reaction has not yet been demonstrated. Therefore, the point of the problem would be to know whether an anatomic cell lesion is produced in the sensitized animal by the direct attack of the antigen introduced again into the organism. Moreover, there also remains open the question whether all cells are sensitized on the injection of a foreign protein or whether only the elements of certain organs or tissues react to the antigenic substance.

Opie¹ suggested that the cells of a sensitized animal could be attacked and injured as the result of coming in contact with the antigen. He admitted that in the Arthus phenomenon the damage of tissue was due to the toxic effect of the precipitate formed by the interaction of protein and the serum of the sensitized organism.

Later Klinge,² injecting protein intravenously in organs isolated from rabbits previously treated, observed phenomena of necrosis in the lymphoid follicles of the spleen. This observation was followed by the experiments of Meyer and Lowenthal.³ These authors made cultures of splenic tissue and lymph nodes of guinea pigs that had been sensitized by horse serum, in a medium containing this antigen, but could not observe any lesion or disturbance of the growth of the fibroblasts or the reticuloendothelial elements. Identical results were obtained with cultures of endothelial tissue. They also obtained normal cultures with organs of animals which had died from anaphylactic shock.

From the Department of Pathology, Instituto Biológico

1 Opie, E. L. *J. Immunol.* **9** 259, 1924

2 Klinge, F., cited by Doerr. *Ztschr. f. Hyg.* **118** 623, 1936

3 Meyer, K., and Lowenthal, H. *Ztschr. f. Immunitätsforsch.* **54** 420, 1927.

Contrary to these observations, Aronson⁴ referred to the results obtained by Sereni and Garofolini, who had noted cell degeneration and death when small quantities of horse serum were added to the culture medium of explants of spleen, bone marrow and omentum of sensitized chicks. Afterward observations were made by Rich and Lewis⁵ on allergy in tuberculosis. A clearly noxious effect of tuberculin was observed on cultures of washed blood and splenic cells. Working with transplants of spleen and bone marrow, Aronson confirmed these results. On the other hand, he admitted the possibility that the mechanism of the reaction to tuberculin may be different from the mechanism of anaphylactic shock and of the Arthus phenomenon. More recently Rich and Follis⁶ reported that there was no reactivity of individual cells in that allergic phenomenon.

The present work deals with experimental results which indicate morphologic changes and death of certain cells of the organism consequent to hypersensitivity reactions. It was apparent that elements of the reticuloendothelial system were damaged and killed when a foreign protein of nonbacterial provenience was inoculated in an animal previously sensitized by this same substance.

In previous experiments it had been noticed that intraperitoneal inoculation of normal horse serum into guinea pigs sensitized by this antigen produced a state of anaphylactic shock, symptomatologically of the protracted type. Within approximately five to ten minutes after the intraperitoneal injection of 2 cc of serum, symptoms of shock develop in these animals, such as emission of feces and urine, cough, scratching of the nose and signs of agitation, followed afterward by a manifest state of somnolence and ill feeling. Then the signs of depression increase, and there is progressive lowering of temperature, with puppling of the ears. The evolution of the symptoms is not rarely followed by death one hour or more after the inoculation of the antigen. Other animals, after the development of identical symptoms, slowly and progressively become normal again within two or three hours.

The animals which had died from shock displayed organic changes, such as slight pulmonary emphysema, marked congestion of the liver, sometimes regional circulatory disturbances or acute tumefaction of the spleen, severe hemorrhage in the gastric mucosa and hemorrhage in the subserosa of the intestine especially in the colon.

These observations were the basis of the present investigations. We started from the idea that probably in this kind of shock the antigen is kept in long and intimate contact with the tissues.

⁴ Aronson, J. D. *J. Immunol.* **25** 1, 1933.

⁵ Rich, A. R., and Lewis, M. R. *Bull. Johns Hopkins Hosp.* **50** 115, 1932.

⁶ Rich, A. R., and Follis, R. H., Jr. *Bull. Johns Hopkins Hosp.* **66** 106, 1940.

EXPERIMENTAL PROCEDURE

Guinea pigs weighing about 250 Gm were sensitized by injection of 1 cc of a 1:10 dilution of normal horse serum in saline solution. From twenty to twenty-two days after the sensitizing injection, the shock was brought about by means of an intraperitoneal injection of 2 cc of horse serum. All the guinea pigs displayed definite symptoms of shock, and some of them died within one hour after the shocking injection. From the animals which died, fragments of liver, spleen and lymph nodes were collected immediately, placed in small sterile flasks containing Tyrode's fluid and kept in the incubator at 37 C. The other animals, which survived, were killed after one hour, and material was collected in the same way under the same conditions.

The material was kept in the incubator for five, six, seven, eight, ten, eleven, eighteen and twenty hours, then taken out and fixed with Heidenhain's fluid for three or four hours, embedded in paraffin and stained with hematoxylin-eosin.

In another series the animals were killed two, three, five, six, seven, eight and nine hours after the onset of the shock and their tissues immediately fixed with Heidenhain's fluid.

The control animals were not sensitized but received 2 cc of serum intraperitoneally.

Eighty-three guinea pigs were used, 25 of which were controls.

OBSERVATIONS

Liver—The liver of sensitized animals that died from shock or were killed approximately one hour after the injection did not show significant changes. Only a slight enlargement of the nuclei of the endothelial cells of the sinusoids could be observed. However, in the sensitized animals that were killed after three or more hours, changes as described later were evident. This was true also in the surviving tissue prepared according to the technic just described. It was possible to note that the state of nuclear tumefaction observed at first was only the initial phase of a heavy damage of the Kupffer cells. These slight initial manifestations developed within about three hours after the initiation of shock. In some cases numerous cells showed the nucleus in a stage of extreme tumefaction, resembling a bladder or a balloon, and surrounded by a thickened, clearly visible membrane. The impression obtained was that the substances contained in the nucleus were pressed close to the membrane, thus giving the picture of peripheral hyperchromatosis, which I like to call "shell nucleus." Such a change seems to be incompatible with the cell's life, the phenomenon is irreversible, and this stage is probably followed by a phase of disintegration. As a result, either signs of lysis of the thickened membrane or the striking picture of nuclear rupture can be observed. It could be verified that, probably owing to an excessively high internal pressure, the rupture of the membrane sometimes occurs, the content being expelled. The nucleus then gets a shriveled appearance and finally disintegrates.

These phenomena could be distinctly observed from three to seven hours after the occurrence of the shock. It must be emphasized, however, that such intense alterations were found quite rarely, generally the lesion observed was of the same type but more discrete, although ending with nuclear lysis. Even no cell reaction was observed in some cases. On the other hand, the lesion was not generalized among the elements of the same organ, in many cases practically resting, normal cells were seen among others with more or less marked reaction.

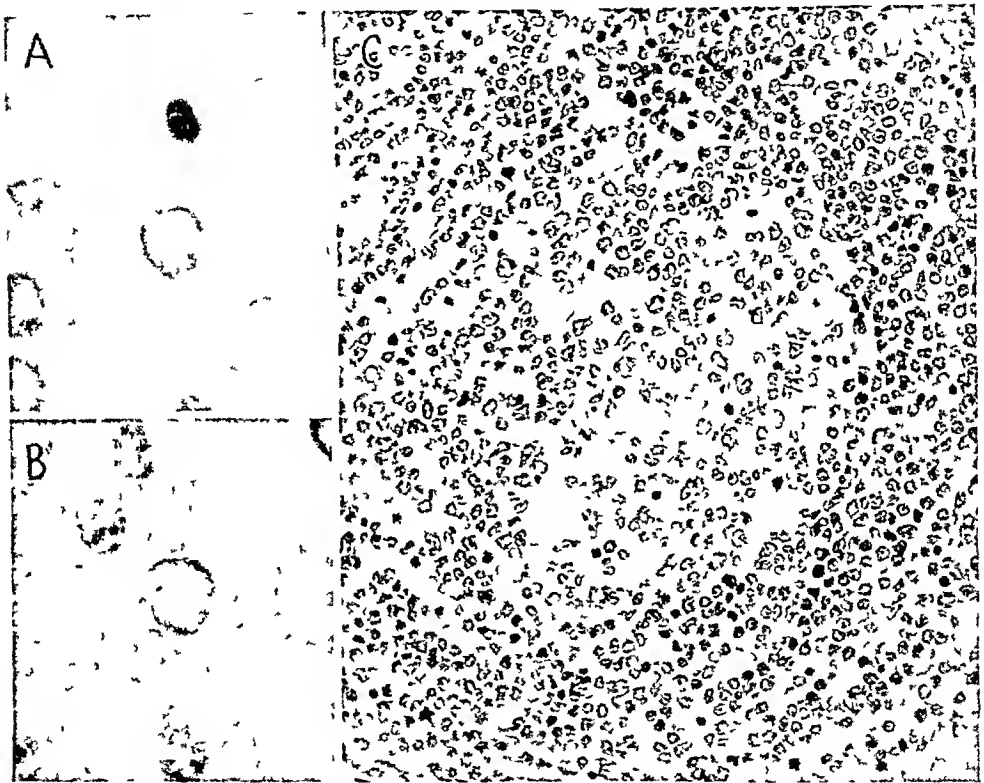


Fig 1—A, liver fixed five hours after the shock-inducing inoculation. Kupffer cell with heavy tumefaction of the nucleus beside an element which is at rest $\times 1050$, Zeiss objective.

B, liver maintained in the incubator for six and a half hours (eight and a half hours after the shock-inducing injection). The Kupffer cell's nucleus has a bladder-like aspect. Compare it with the nuclei of the hepatic cells $\times 1050$, Zeiss objective.

C, spleen fixed five hours after the shock-inducing injection. A lymphoid follicle is shown with numerous cells in a state of necrobiosis $\times 280$, Zeiss objective.

Only certain changes, probably of secondary significance, could be noted in the liver cells. They appeared larger and more turbid and showed less affinity for stains than normal liver cells.

In control animals, not previously sensitized, the intraperitoneal injection of serum did not cause the development of a picture similar to that described. The material of these animals, studied under the

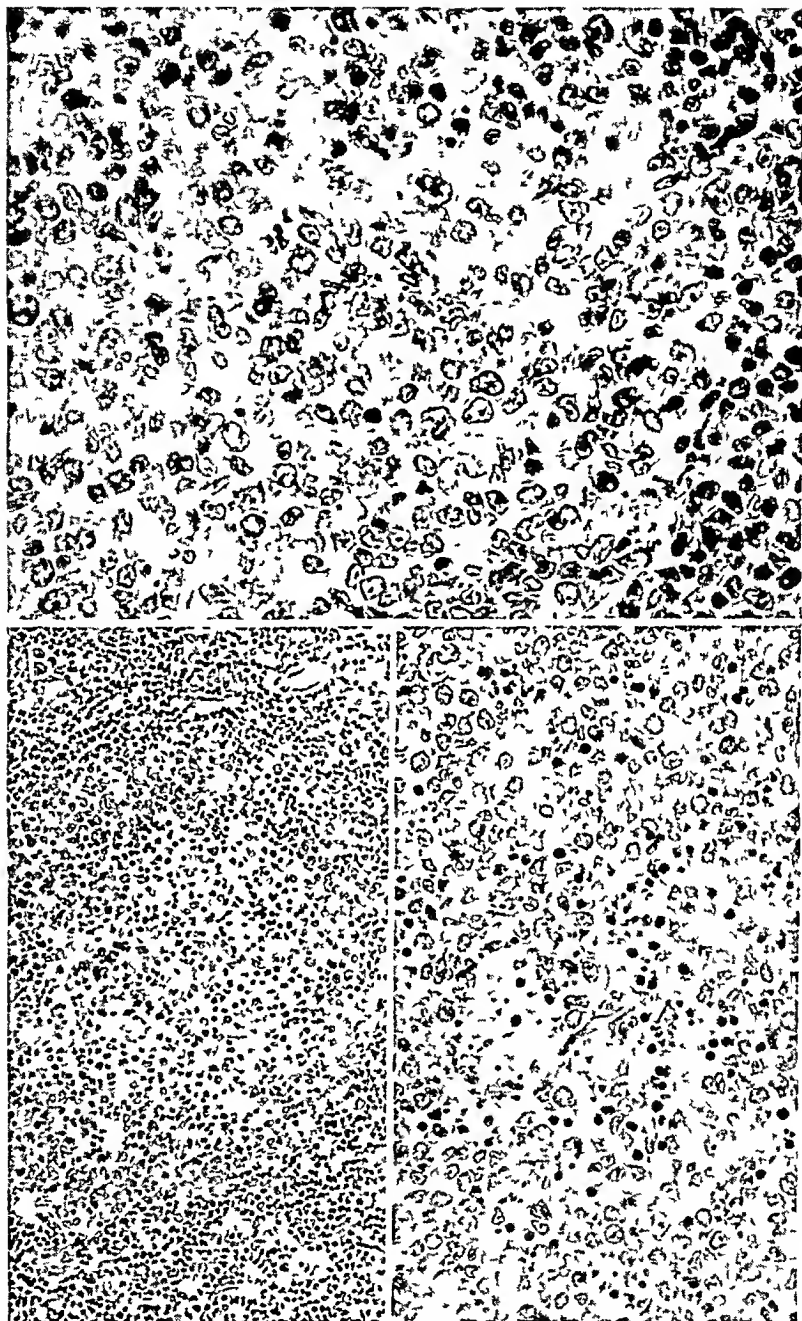


Fig 2—*A*, spleen fixed seven and a half hours after the shock-inducing inoculation. A secondary follicle is shown. Note disintegrating cells and cell particles $\times 450$, Zeiss objective.

B, lymph node fixed seven hours after the shock-inducing injection. A secondary follicle is seen, distinctly rarefied $\times 150$, Zeiss objective.

C, lymph node fixed eight and a half hours after the shock-inducing inoculation. A secondary follicle with numerous disintegrating elements is shown. Nuclear pyknosis $\times 320$, Zeiss objective.

same conditions, showed only a slight state of engorgement of the Kupffer cells, and occasionally one or another element attained a more advanced phase of tumefaction without showing any change in its structure

At this point I must refer to a detail important for these observations. The technic of maintaining the animal alive for varying lengths of time could not be employed throughout all the successive studies of cell changes, for at the beginning a marked decrease in the number of recognizable Kupffer cells was evident in animals killed four or five hours after the occurrence of the shock. (The combined use of both technics appeared therefore of advantage.)

These elements after being altered were possibly drawn along by the circulation, only the undamaged cells being kept by the liver. If the process of maintaining the organ *in vitro* is used, that inconvenience is avoided, the cells being "in loco" for further observation.

Spleen—Cell changes were also noted in the spleen, but only in the cells of the germative centers of the follicles. These lesions were observed in various phases. Examination with low magnification showed in these cases an evident change in the structure of the follicles, the germative centers appeared clearer than usual and as if they were rarefied. With higher magnification a distinct cell reaction could be perceived. The cells of the germative centers seemed more intensely damaged. Cells with large and clear nuclei were scantily scattered throughout the follicle. The nuclear enlargement was of the same extent as that in the Kupffer cells and was also associated with nuclear hydrops and signs of peripheral hyperchromatosis. In later stages lysis of the membrane or signs of nuclear rupture were also noted. Some of the cells displayed distinct pyknosis, others, karyorrhexis and karyolysis.

Finally, I must refer to the absence of reaction of the reticuloendothelial cells of the red pulp. In these elements only slight enlargement of the nucleus was observed.

In the control animals no changes of the lymphoid follicles were observed.

Lymph Nodes—Submaxillary, retropharyngeal, prescapular and inguinal nodes were studied. Cell changes of varying intensity were frequently observed, mainly in the follicular and extrafollicular germative cells of the cortex.

The large and clear follicular cells and the young reticular elements scattered throughout the cortical layer were most intensively damaged. Usually the most extensive changes were present in the secondary follicles. The picture of cell reaction, with distinct enlargement of the nucleus, was identical with that described in the spleen. Cells which

seemed to be lymphoblasts showed a different kind of reaction, leading to pyknosis and nuclear fragmentation

No reaction could be observed in other reticuloendothelial elements of the lymph nodes, neither the endothelial cells of the lymphatic sinuses nor the reticular cells of the medullary cords showed any important sign of damage of their structure

In the control animals the follicles and other portions of the cortex and the medulla showed no significant changes

COMMENT AND SUMMARY

The cell changes described might be interpreted as a consequence of the antigen-antibody reaction in the organism of the sensitized animal. Lesions have been observed only in a certain type of reticuloendothelial cells, mainly in the Kupffer cells. The significance of this observation cannot be definitely ascertained at the present time. It may be, however, more than a coincidence that the only type of cells found injured after the shocking injection were those which have been credited with playing a role in the formation of antibodies, namely the reticuloendothelial⁷ and the lymphoid cells⁸

7 Sabin, F. R. *J. Exper. Med.* **70** 67, 1939

8 Harris, T. N., Grim, E., Mertens, E., and Ehrlich, W. E. *J. Exper. Med.* **81** 73, 1945

CAROTID-CAVERNOUS FISTULA

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THE INTERNAL carotid, after it has passed through the carotid canal in the petrous portion of the temporal bone, enters into the cavernous sinus. Here the artery is adherent to and extends along the entire length of the lateral wall of the sinus. The artery is separated from venous blood only by the endothelial lining of the sinus. The cavity of this sinus, which is irregular in shape and size, is so divided by numerous fibrous strands that it assumes the appearance of cavernous tissue.¹ The oculomotor, the trochlear, the ophthalmic and the abducens nerves and the gasserian ganglion are also within the sinus. It has been known for many years that the portion of the internal carotid artery within the cavernous sinus occasionally ruptures. This lesion is most frequently described under the title "Pulsating Exophthalmos."

Travers² was apparently the first to describe this condition, in 1809. Since that time more than 800 cases have been described, and in 1943 Martin and Mahon³ studied the entire literature subsequent to Locke's⁴ extensive review in 1924. From the standpoint of pathology, however, relatively little has been written on the lesions present in patients with this condition. Baron⁵ was the first to perform an autopsy in which a fistula between the internal carotid artery and the cavernous sinus was demonstrated. Subsequently, additional cases examined at autopsy have been described, and these have been reviewed by Dandy.⁶ He stated that up to 1937 29 postmortem specimens had been described, most of them in foreign literature. Since that time Bergstrand,⁷ Sugar and Meyer⁸ and Cunningham⁹ have each reported an additional case. Dandy and Folles¹⁰ have written an excellent article devoted mainly to the pathologic aspects of carotid-cavernous fistula, in which they describe 2 additional cases.

1 Young, A. H., and Robinson, A., in Cunningham, D. J. Cunningham's Text Book of Anatomy, ed. 6, New York, Oxford University Press, 1931, p. 989.

2 Travers, B. Med-Chir. Tr. **2** 1, 1813.

3 Martin, J. D., Jr., and Mahon, R. F. J. A. M. A. **121** 330, 1943.

4 Locke, E. C., Jr. Ann. Surg. **80** 1, 1924.

5 Baron. Bull. Soc. d'anat. de Paris, 1835, p. 178.

6 Dandy, W. E. Zentralbl. f. Neurochir. **2** 77, 1937.

7 Bergstrand, H. Acta radiol. **18** 58, 1937.

8 Sugar, H. S., and Meyer, S. J. Arch. Ophth. **23** 1288, 1940.

9 Cunningham, J. C. J. Iowa M. Soc. **32** 495, 1942.

10 Dandy, W. E., and Folles, R. H. Am. J. Ophth. **24** 365, 1941.

A fistulous opening in the internal carotid artery within the cavernous sinus is undoubtedly the most common cause of "pulsating exophthalmos." Nevertheless, it should be pointed out that other lesions can be associated with this syndrome. Occasionally other lesions of the internal carotid artery are found. These include aneurysms outside the cavernous sinus,¹¹ also arteriovenous aneurysms, with the jugular vein most often found in the carotid canal. In a few instances an aneurysm of the carotid artery in the cavernous sinus without evident rupture⁹ has been found with pulsating exophthalmos. In such cases it is possible that a minute opening may have existed during life. Pulsating exophthalmos may be the result of intraorbital lesions. These include simple aneurysms of the ophthalmic artery, intraorbital arteriovenous aneurysms and, more rarely, other intraorbital tumors. Other causative conditions are meningocele, absence of a large part of the orbital roof, intracranial tumors and other tumors extending into the orbit.¹²

In approximately 75 per cent of cases of pulsating exophthalmos trauma is considered to be the important etiologic factor. It is to be expected that in virtually all of these there will be a carotid-cavernous fistula, and Locke⁴ found such a fistula in 16 of 17 cases examined by autopsy. Usually a fairly severe blow to the head precedes symptoms by several or many hours. The internal carotid artery, as well as the cavernous sinus, is immobile, and a sharp blow may cause rupture of the artery with or without fracture of the adjacent sphenoid bone. Arteriosclerosis of the artery increases the likelihood of rupture in such cases. Nevertheless, ruptures can occur in young people in whom little or no sclerosis is observed.

Approximately 25 per cent of cases of pulsating exophthalmos are nontraumatic, and only about half of these have carotid-cavernous fistula as the cause. The most important etiologic factor in this group is sclerosis of the internal carotid artery. Parry and Rogers¹³ reported that a patient of theirs had aneurysm formation after violent sneezing, hence increased blood pressure may be an added factor. Undoubtedly, another factor is of importance in these situations, since it is most unusual for spontaneous openings to form in medium-sized arteries. The anatomic relationships here are unique, and it may well be that lack of tissue support of the internal carotid artery within the sinus increases the likelihood of the formation of a fistulous opening. Although some stress has been given to the position and the size of these openings in regard to severity of exophthalmos, Dandy and Folles¹⁰ expressed the belief that no definite relationship exists. That some fistulas are

11 Ellis, N. B. *Texas State J. Med.* **35** 483, 1939.

12 Harkness, G. F. *Internat. J. Med. & Surg.* **43** 243, 1930.

13 Parry, R., and Rogers, L. *Brit. J. Surg.* **27** 179, 1939.

the result of ruptured "berry" aneurysms is possible but may be difficult to prove. Dandy and Folles¹⁰ in 1 case found such aneurysms in the circle of Willis and postulated that the opening within the sinus may have been the result of rupture of an aneurysm. According to Dandy,⁶ no case of proved congenital arteriovenous aneurysm has been found at autopsy. Although syphilis has been suspected, it apparently has not been found at postmortem examination.⁴ As would be expected, the average age of the patients with spontaneous fistulas is approximately fifteen years higher than the age of those whose aneurysms are produced by trauma.⁴

The primary lesion of a carotid-cavernous aneurysm is an opening in the wall of that portion of the internal carotid artery which is within the cavernous sinus. These lumens have been found to vary from 1 or a few millimeters to actual absence of the artery over a distance of a centimeter or more. More than one opening is occasionally found. Frequently there is ectasia or an actual aneurysm of the artery. Occasionally, abnormal twists are found. In cases produced by trauma little sclerosis may be present. Microscopically, there is a variable degree of intimal fibrosis and thickening, often with cholesterol deposits and calcification. The media is usually definitely thinned, and elastic stains show extensive irregularity and fraying of the internal elastic lamina and other elastic layers with focal areas of complete destruction. Usually little inflammation is noted.

The cavernous sinus is likely to be markedly distended by blood, and the various normal septums are consequently distorted or destroyed. Mural thrombi may be present within the sinus. The intraorbital veins are usually also greatly distended. The intercavernous, the basilar and the superior and inferior petrosal sinuses may all be expanded. Not infrequently the opposite cavernous sinus is dilated as well. This occurs particularly in those cases in which the intercavernous sinus is large and widely patent. The nerves within the sinus are usually flattened or may be interrupted. The gasserian ganglion is elevated and pushed laterally, depending on the amount of distention of the sinus. The pituitary gland and the optic nerves may be compressed, and in some cases atrophy of the latter is found.

The pathologic changes found in the brain vary greatly. Frequently, but not always, free blood is found in the subdural space. Occasionally also there is subarachnoid hemorrhage, which may be prominent over the cerebellum.¹⁰ Sugar and Meyer⁸ reported intraventricular hemorrhage as well as intracerebellar hemorrhage in their postmortem specimen. There can be extensive encephalomalacia involving most of one hemisphere as observed by Karplus.¹⁴ Cunningham⁹ noted encephalomalacia

14 Karplus, P. *Wien klin Wchnschr* 13 357, 1900

with hemorrhage Dandy and Follis¹⁰ reported petechiae within the cortex of the frontal lobe in 1 case, but without change in the ganglion cells In their other case there was encephalomalacia due to old infarcts involving the right frontal lobe and basal ganglions (the fistula was also on the right) A more recent infarct was found involving both cerebral peduncles, and petechiae were present in the island of Reil Additional recent infarcts were also noted within the pons It is evident therefore that lesions within the brain may be minimal or abundant and may or may not be directly associated with the cause of death

The mechanics of the circulation following rupture of the internal carotid artery within the cavernous sinus are relatively simple There is a transfer of arterial pressure to the sinus, which then becomes dilated with rupture of its various septums The blood flow reverses within the superior ophthalmic vein, which begins to dilate and pulsate Smaller orbital veins become similarly involved This results in pulsating exophthalmos and frequently edema and even hemorrhage within the tissues about the eye The petrosal sinuses probably drain away most of the blood from the cavernous sinuses The nerves within the cavernous sinus and the optic nerve may be stretched or compressed, hence loss of vision and extraocular palsies may occur Further complications within the vascular system frequently set in Sattler¹⁵ in a study of 235 cases found bilateral pulsating exophthalmos present in 17 per cent In these cases it is postulated that either by way of the intercavernous or the basilar sinus blood is forced into the opposite cavernous sinus This has been substantiated at autopsy¹⁰ The intercavernous sinus is the most direct connection, and when it is relatively large, bilateral exophthalmos is apt to develop readily In other cases this sinus apparently widens gradually, and exophthalmos of the opposite side develops a week or more later In view of the usual presence of an intercavernous sinus it is of interest that in most cases pulsating exophthalmos is not bilateral This can be explained only on the basis that the connecting channels are small or absent A few cases of contralateral exophthalmos have been observed—for instance, that reported by Geis¹⁶ and that by Dandy and Follis¹⁰ The latter was proved by autopsy The lack of ipsilateral exophthalmos was found to be due to a thrombus of the ophthalmic vein The intercavernous sinus was widely patent, and the opposite cavernous sinus was also greatly dilated Another possible cause of this condition might be congenital absence of the ipsilateral superior ophthalmic vein with a widely patent intercavernous or basilar sinus It is of interest that in the case observed by Dandy and Follis bilateral exophthalmos was noted in a previous

15 Sattler, C H, cited by Dandy⁶

16 Geis, F *Klin Monatsbl f Augenh* 106 209, 1941

hospital visit Thrombosis therefore apparently cured the exophthalmos on one side

REPORT OF A CASE¹⁷

Mrs L G, a 61 year old white woman, was admitted to the hospital in coma. She had become suddenly comatose about twenty hours before admission. She was incontinent but moved her limbs freely. Previous to this the patient had several periods of unconsciousness from which she recovered in about five minutes. This had been noted for approximately six weeks. There were also gradual loss of hearing, aphasia and change in personality. The past history revealed that the patient had a "stroke" in her left eye several months before. In addition, she had a cataract removed from the right eye approximately seven weeks previously.

The patient was comatose, with dry, loose skin. There was some hyperemia of the conjunctivas. The right eye showed an irregularity of the iris, presumed to be due to the previous operation. No exophthalmos was noted, and both eyeballs had normal tension. There was some dilatation of the left pupil, and no response to light was noted. The neck was slightly stiffened. No significant changes were noted in the thorax and the abdomen. There was no paralysis of the extremities, Babinski's reflex was not present, and the other reflexes were average. The blood pressure varied from 125 systolic and 55 diastolic to 124 systolic and 90 diastolic.

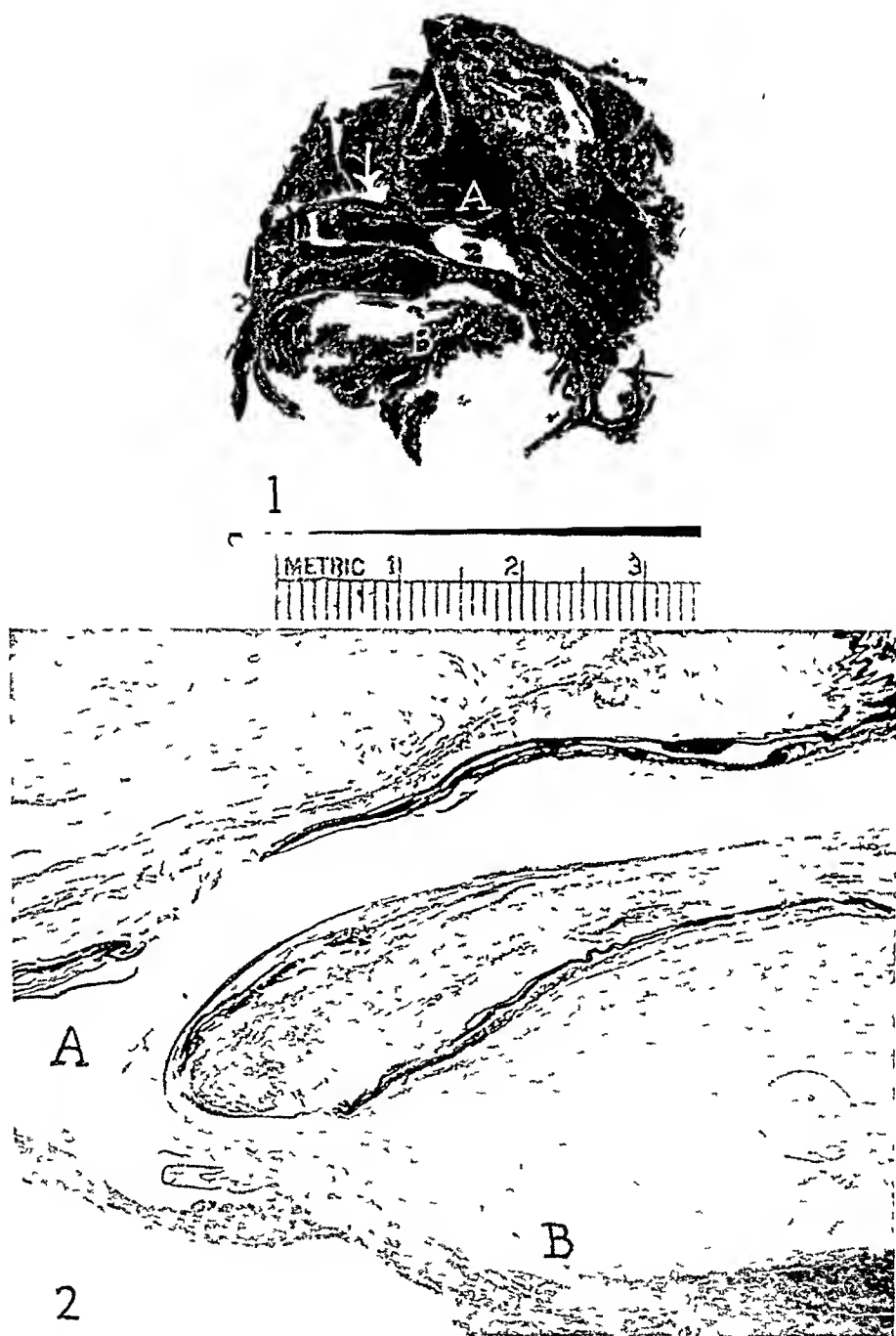
Urinalysis revealed albumin (2 plus) and a moderate number of leukocytes in the sediment. The red blood cell count was 3,600,000 and the hemoglobin value 11.9 Gm, the white blood cell count was 9,800. The blood urea was 42 mg and the blood sugar 106 mg per hundred cubic centimeters. The spinal fluid was clear, with a 4 plus precipitate in the Pandy test. The Nonne-Appelt phase I was 2 plus, and phase II was 3 plus. The protein was 67 mg per hundred cubic centimeters of fluid. The mastix test was 444420, and the Kline exclusion and diagnostic tests were negative.

The patient partially regained consciousness the day after admission and for several days was able to answer questions slowly and move her extremities. Gradually her condition became worse, and she died on her sixteenth hospital day.

Autopsy—The only external features of note were medial deviation of the left eyeball and dilatation of the left pupil (9 mm) and an irregularity of the right pupil, presumably due to a previous operation. No proptosis was noted. The heart weighed 200 Gm and was moderately dilated. There was marked calcification of the cusps of the aortic valve with formation of an acquired bicuspid valve. Only slight thickening without deformity of the mitral and tricuspid leaflets was noted. The coronary arteries were only slightly sclerotic. The aorta showed marked sclerosis. The lungs were hyperemic and emphysematous. There was an obsolete primary tuberculosis complex involving the upper lobe of the left lung and the corresponding bronchopulmonary lymph node. The liver and the spleen were hyperemic and contained obsolete miliary tubercles. The gallbladder contained several calculi. The pancreas, the adrenal glands and the genitourinary tract showed no significant changes except chronic cystitis (cystitis lymphomatosa). There were acute esophagitis and diverticulosis of the sigmoid colon.

The brain weighed 1,250 Gm. There was no subdural or subarachnoid hemorrhage. The leptomeninges were of average thickness, and the gyri and the sulci had the usual configuration. The base of the brain about the tuber cinereum,

¹⁷ This case report is made with the permission of Dr Gannon and Dr J F Slowey



1, photograph of the carotid-cavernous fistula. A black arrow shows the carotid artery cut longitudinally and a white arrow the proximal part cut transversely. At A is the opening in the carotid artery, showing the slightly rounded margin. At B is the thrombus in the cavernous sinus.

2, photomicrograph ($\times 31$) showing a longitudinal section of the internal carotid artery. At A is the opening corresponding to A in 1. Notice also the fraying and interruption of elastic fibers. At B is the thrombus in the cavernous sinus.

especially to the left, was compressed somewhat by an underlying mass. The optic tract and nerves, as well as the left third, fourth and sixth cranial nerves, were moderately distorted by the same mass. The circle of Willis was complete, and the vessels were of moderate size, showing moderate to marked sclerosis without significant narrowing of the lumen. Similarly the intracranial portions of the internal carotid arteries showed marked sclerosis but without marked lessening of the lumens. Cross section of the brain failed to reveal any gross lesions in the brain substance. The ventricular system was of average size and contained no blood. Microscopically, slight edema of the brain substance was noted, with slight degenerative changes in some of the neurons. These were nowhere severe in degree and were interpreted to be the result of partial ischemia of the brain. No hemorrhage or necrosis was observed, although many sections were taken from all principal portions of the brain.

The left cavernous sinus was greatly distended, measuring 3 cm superoinferiorly, 3.2 cm anteroposteriorly and 4.2 cm mediolaterally. Its external surface was gray and without hemorrhage. The medial portion extended into the sella turcica approximately 5 mm beyond the midline. The pituitary gland was raised and compressed. The entire optic chiasm was elevated and pushed anteriorly, with distortion particularly of the left optic nerve. Some distortion of the third, fourth and sixth cranial nerves was also noted. The internal carotid artery at its exit from the cavernous sinus was anterior and to the right. Section revealed the enlarged sinus to be filled with clotted blood, which in the inferior and anterior portion was firm, laminated and adherent to the endothelium. The various septums were completely obliterated. The area about the region of the superior ophthalmic vein was completely covered by adherent thrombus. The orbital veins were unfortunately not examined. No portion of the circular sinus could be identified, and the basilar and petrosal sinuses were not dilated. The right cavernous sinus was also not enlarged.

In its course through the petrous portion of the temporal bone the internal carotid artery was of average size. Although there were many intimal plaques, the lumen was only slightly decreased in size. No external variations were noted in the foramen lacerum medium or at the entrance into the cavernous sinus. Within the sinus the artery widened slightly from its average diameter of 5 mm up to 8 mm. Then its wall became frayed and friable and for a distance of 1 cm it was completely unrecognizable. At a point 8 mm from its exit the superior portion of the wall was recognizable, and at the exit it was complete and the lumen was of a diameter similar to that at its entrance.

Microscopically, the wall of the cavernous sinus was composed of collagenous connective tissue. This contained a few foci of hemorrhage and small collections of lymphocytes. A lining endothelium could be demonstrated only in some places. There were adherent thrombi in some areas, but these showed little organization. There was moderate to marked intimal fibrosis and thickening of the internal carotid artery. Its media showed extensive degeneration and actual necrosis. In one area the media had a myxomatous appearance with a neutral stain, but no actual cyst formation was noted. No inflammation was noted. Considerable pyknosis of nuclei was found in some areas. Elastica stains revealed extensive fraying and degeneration of elastic tissue, particularly of the internal elastic membrane, and this was especially marked at the edge of the ruptured portion. In one section the elastica of the artery was slightly reflected over the lining of the cavernous sinus.

COMMENT

At no time in the hospital was this patient noted to have exophthalmos, hence the true nature of the underlying lesion was not clinically suspected. Certainly, to exclude exophthalmos completely, accurate measurements must be taken, nevertheless, if any was present in this case, it was extremely slight. Both eyes were palpated, and no pulsation was noted. Unfortunately, no attempt was made to hear a bruit. It is surprising, however, that the patient did not complain of noise within her head, for this is a frequent symptom. One lone statement in the history gives a clue indicating that the fistula originated about two months before the patient's admission to the hospital—namely, the statement that the patient had a "stroke" in the left eye at that time. Just what this "stroke" was is not known, but it is possible that she had an actual pulsating exophthalmos at that time which later receded because of thrombosis of the ophthalmic vein. This vein was not examined, because even at the time of autopsy the true nature of the lesion was not suspected. Nevertheless, the thrombus in the anterior and inferior area of the sinus suggests that the vein also was occluded by a thrombus.

Undoubtedly, arteriosclerosis was the most important factor causing rupture of the artery. In a few areas the changes resembled somewhat those found in idiopathic medionecrosis of the aorta, and this may possibly be an additional etiologic factor in the present instance. The defect in the artery was much larger in this than in virtually all previously recorded cases. The cavernous sinus was also dilated more than in some previous cases, although in not many cases is there a record of accurate measurements. The absence of dilatation of the right cavernous sinus can be explained only by absence of, or occlusion by distortion of, the intercavernous sinus and by absence of dilatation of the basilar sinus. The immediate cause of death was undoubtedly a combination of dilatation of the heart and ischemia of the brain. It is of interest that no hemorrhage or infarct was found anywhere within the brain.

SUMMARY

A patient with a carotid-cavernous fistula in whom no pulsating exophthalmos was noted came to autopsy. The rupture of the carotid artery was extensive. No infarct or hemorrhage was found in the brain.

TRUNCUS ARTERIOSUS COMMUNIS PERSISTENS

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THERE is no one typical picture of truncus arteriosus communis persistens. Humphreys¹ in 1932 made a careful study of the developmental factors concerned and formulated criteria for the identification of this rare anomaly. These criteria are as follows:

- 1 Only one large arterial trunk leaves the base of the heart. This is the primary requirement.
- 2 The arterial trunk must combine the features and the functions of both the aorta and the pulmonary artery, on the one hand giving off the coronary and systemic arteries, and on the other supplying blood to the lungs.
- 3 The interventricular septal defect is always present.
- 4 The trunk is commonly in the position of the so-called rider aorta with varying degrees of shift to the right.
- 5 The surest landmark of the common trunk is its four semilunar valves.
- 6 The crista supraventricularis proper should stop at the base of the septum, beneath the ostium of the trunk.
- 7 There is patency of the foramen ovale, varying in degree from case to case.
- 8 The mitral and the tricuspid leaflets undergo modifications of form and attachment according to the degree of shift of the trunk to the right.

The necessity of four cusps is not accepted by Abbott,² Hunter³ and Lev and Saphir.⁴ They require that the large single vessel give rise only to the coronary, pulmonary and systemic vessels.

Before a final diagnosis of persistent common arterial trunk can be made, it is necessary that pulmonary or aortic atresia be rigidly ruled out (Abbott²). These atresias occur in cases of truncus solitarius aorticus or pulmonalis, an anomaly in which one trunk functions for both cir-

1 Humphreys, E. M. Arch Path **14** 671, 1932

2 Abbott, M. E. Atlas of Congenital Cardiac Disease, New York, American Heart Association, 1936

3 Hunter, O. B., Jr. Arch Path **37** 328, 1944

4 Lev, M., and Saphir, O. J. Pediat **20** 74, 1942

culations, the other is obliterated, and may be found as a fibrous remnant attached to the base of the heart

Only 37 of the cases recorded in the literature conform to the foregoing criteria Abbott⁵ in 1927 recognized 23 cases, but in Humphreys'¹ review, published in 1932, only 15 were listed as acceptable Of these, 5, including 1 of her own, had the theoretically required four semilunar cusps, 3 cases had three cusps and a rudimentary fourth cusp, 7 cases had only three cusps but satisfied many of the other requirements In 1942 Lev and Saphir⁴ compiled 19 additional cases Other recent contributions were those of Van Brown,⁶ Doerr⁷ and Hunter,³ each of whom reported 1 case The case now reported is the thirty-eighth

REPORT OF A CASE

A boy was born in Provident Hospital May 1, 1945 The mother, a secundipara, was 17 years old Her serologic tests were negative The first child was living and well Gestation and delivery were uneventful The newborn infant's weight was 2,770 Gm Because of cyanosis and difficulty in breathing, he was placed in an oxygen tent On the ninth postnatal day a roentgen examination of the chest showed "no significant lesion" Cyanosis and dyspnea continued On the seventeenth day he was discharged, "not much improved"

He was readmitted November 27, acutely ill, with a history of a severe cold that began ten days previously On admission his temperature was 97 F Four hours later it had fallen to 96.4, it then rose rapidly to a peak of 100.4, after which there was a sharp decline to 98.8 The significant physical findings were marked cyanosis, thick whitish fluid in the throat, purplish discoloration of the oral mucosa, harsh bronchial breathing and a hyperdynamic heart, enlarged to the right The clinical impression was acute tracheobronchitis and congenital heart disease

Therapy consisted of the administration of oxygen, nikethamide fluids (parenterally administered) and penicillin There was no apparent impression on the course of the disease, and death occurred fifteen hours after the infant was admitted to the hospital

Autopsy (thirty-three hours after death)—The anatomic findings, exclusive of those in the heart, were as follows The body was that of a poorly nourished infant boy, 62 cm in length and 4,750 Gm in weight The skin was loose and wrinkled The head was symmetric and covered with soft, thin dark brownish hair The posterior fontanel was closed, the anterior fontanel was patent There were no erupted teeth The subcutaneous, omental and mesenteric fat depots were depleted The stomach and the intestines were distended with gas There was no free fluid in the abdominal cavity The peritoneal surfaces were smooth and moist The lower border of the liver was 2 cm below the costal margin in the right mid-clavicular line and 1.5 cm below the xiphoid process in the midline The liver was pale brownish and firm in consistency Its weight with the gallbladder was 185.3 Gm The other abdominal organs were in their normal positions and showed

5 Abbott, M. E. Congenital Cardiac Disease, in Osler, W., and McCrae, T. Modern Medicine, Philadelphia, Lea & Febiger, 1927, vol. 4, p. 612

6 Van Brown, D. J. Tech. Methods **22** 101, 1942, cited by Hunter³

7 Doerr, W. Virchows Arch f. path. Anat. **310** 304, 1943, cited by Hunter³

no significant gross changes. There was no free fluid in the pleural cavities. The pleural surfaces were smooth and moist. The thymus extended from the thoracic inlet to the ventral surface of the pericardium, to which it was attached. Its weight was 5.3 Gm. The mucosa of the trachea and the main bronchi was hyperemic. These passages contained thick, frothy, grayish white fluid. The left lung weighed 46 Gm. The cut surfaces of both lungs showed patchy, irregular areas, varying in color from grayish yellow to dark red, characteristic of bronchopneumonia.

The heart was approximately in the center of the thorax. The transverse dimension of the pericardium was 7 cm. The right border was 4 cm and the left border 3 cm from the midsternal line. The pericardial sac contained 2 cc of thin amber fluid. Its inner surfaces were smooth and moist. The external surface



Persistent truncus arteriosus communis anterior view showing the enlarged right ventricle, the single trunk, the patent ductus arteriosus and the septal defect with glass rod inserted.

of the heart was smooth and dull red. The coronary vessels were prominent and slightly tortuous. There were a few small deposits of pale yellowish fat along the courses of these vessels. The right atrium was markedly dilated and contained a soft, pale yellowish clot and some dark red fluid blood. The lining of the right ventricle was smooth, thin and transparent. A 3 mm probe was readily passed through the patent foramen ovale. The right atrioventricular ("tricuspid") orifice was 4.2 cm in circumference. It did not have a distinct posterior leaflet. There was a large anterolateral leaflet, as well as a smaller medial leaflet. The anterolateral leaflet was connected by long slender chordae tendineae to the anterior

papillary muscle, which was 3 mm thick. Lateral to this muscle was another papillary muscle, 1 mm thick. It was inclined medially and its chordae were attached to the same leaflet.

The medial leaflet was connected to the interventricular septum by several small chordae and to a papillary muscle, 3 mm thick, which arose from the posterior wall close to the septum. The right ventricle was markedly dilated. Its greatest inner circumference was 4.5 cm, and the distance from the atrioventricular ring to the apex was 3.5 cm. The wall, including the columnae, was hypertrophic, having a thickness of 7 mm. The endocardium was smooth, thin and transparent. The myocardium was dull red and firm in consistency. The interventricular septum was defective in its upper membranous edge. This spheroidal septal defect was 10 mm in its greater transverse diameter and 7 mm in the lesser superior-inferior dimension. In the rider position, immediately above the defect, was the ostium of the one great vessel from the heart. The ostium was 3.5 cm in circumference and was approximately two thirds over the right ventricle. The myocardial fibers of the ventricle apparently terminated in a fibrous cord that encircled the common truncus. The ostium had three well developed cusps. There were two coronary orifices, the right coronary orifice was in the anterior sinus, and the left coronary orifice was in the left posterior sinus. The intima of the common trunk was smooth and whitish, there was no ridge or other evidence of a vestigial septum. The branches of the truncus, in their normal positions, were the coronary, the innominate, the left common carotid and the left subclavian arteries. On the descending portion of the arch, 1 cm from the orifice of the subclavian artery, was the ductus arteriosus with a patent lumen 5 mm in diameter. This vessel had a main stem 5 mm long, which divided into two branches, one entered the hilus of the left lung, and the other extended to the hilus of the right lung. No other vessel was found emerging from the heart, nor the fibrous cord of an atretic vessel. The remainder of the branches of the thoracic and abdominal aorta appeared normal. The left atrium was comparatively small and was connected with the pulmonary veins from both lungs. The mitral valve had two normal-appearing leaflets. Its orifice was 3.2 cm in circumference. The left ventricle had an inner circumference that measured up to 3 cm, and the distance from the mitral ring to the apex was 3.4 cm. Its wall was 6 mm thick at the base of the anterior papillary muscle and 4 mm thick at the apex. The weight of the heart with the attached common trunk and the descending thoracic aorta was 59.3 Gm.

Microscopically, bronchopneumonia, pulmonary edema and congestion, fatty hepatic change and cloudy swelling of the kidneys were observed.

COMMENT

The anatomic features of this case were as follows:

1. There was only one large vessel at the base of the heart. This truncus straddled the interventricular septum and thus emerged from both ventricles, approximately two thirds from the right and one third from the left. Its orifice was guarded by three well developed cusps. The truncus gave off two coronary arteries and the systemic arteries in their normal positions.

2. The aorta and the pulmonary arteries were undifferentiated. It is generally held that the arch of the aorta and its branches, except

the right subclavian, develop from the left member of the fourth pair of aortic arches. The right and left pulmonary arteries develop from the sixth pair of aortic arches. Fusion of the paired spurs of the fourth and sixth aortic arches and their caudal extension form the aortic-pulmonary septum. In this case there was no ridge or septum in the wall of the truncus, indicating the absence of structures derived from the sixth arch. This type of deformity is classified as complete, to differentiate it and the partial type in which some remnant of the aortic-pulmonary septum remains.

3 The pulmonary circulation was supplied by a large vessel which branched from the truncus at the site of the ductus arteriosus. This is a most unusual site for the origin of the pulmonary arteries, and the literature contains a report of only 1 previous case (Hunter³). In other cases the pulmonary arteries have originated as two large branches from the wall of the common truncus some distance above its origin, or as a single vessel which divides, sending a branch to each lung. In the case of Graham and Montgomery⁸ the pulmonary circulation was supplied by two small arteries arising from the truncus distal to the great vessels, apparently these were bronchial arteries. In Kettler's⁹ case the truncus, immediately above the ostium, divided into a larger aortic branch and a smaller pulmonary branch.

4 The interventricular septal defect was in the upper membranous part of the septum. Varying degrees of defectiveness of the septum have been described. It may be rudimentary or absent. The dextro-position may be such that the ostium emerges more or less entirely from the right ventricle, with the blood entering it from the left through the defect. One would expect the interventricular defect to cause a loud systolic murmur to the left of the sternum, but none was recorded in this case.

5 There was a patent foramen ovale. In the Lev-Saphir⁴ tabulation some cases are listed in which the auricular septum was almost or completely absent.

6 The right auriculoventricular valve had only two leaflets. In this respect the case is similar to that of Lev and Saphir⁴. They explained this on the basis that the bulbus was absorbed earlier in fetal life than the formation of the valves and the presence of the large truncus in the right ventricle at the time of the formation of the valves created current relationships similar to those at the mitral valve. The inflow areas of the right ventricle met at a very acute angle, so that development of a third leaflet was inhibited.

⁸ Graham, S., and Montgomery, G. L. *J. Tech. Methods* **81**, 1938, cited by Lev and Saphir⁴.

⁹ Kettler, L. H. *Virchows Arch. f. path. Anat.* **304**, 513, 1939.

SUMMARY

The case of truncus arteriosus communis persistens presented is the thirty-eighth to be recorded and the second in which the pulmonary arteries were branches of a patent ductus arteriosus. Death occurred from malnourishment and bronchopneumonia at the age of 6 months. Only 2 patients with this anomaly have been known to live to adult life. One died at the age of 16 and the other at 25.

There is no conclusive explanation for the anomaly. In the literature are the theories of Spitzer,¹⁰ Humphreys¹ and Lev and Saphir.¹ The anomaly may be associated with abnormal torsion which results in failure or impairment of septal formation. In this case there was aplasia or complete early regression of sixth arch structures.

¹⁰ Spitzer, A. *Virchows Arch f path Anat* **243** 81, 1923, cited by Humphreys¹

ATHEROSCLEROSIS AND ARTERIOSCLEROSIS IN DOGS FOLLOWING INGESTION OF CHOLESTEROL AND THIOURACIL

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SINCE Anitschkow¹ demonstrated in 1913 that lesions similar to those of human atherosclerosis develop in the arteries of rabbits fed large amounts of cholesterol, numerous investigators have attempted to produce experimental atherosclerosis by feeding cholesterol to other animals. However, these efforts have succeeded only with regard to chickens² and guinea pigs³. Attempts to produce atherosclerosis in dogs, cats and monkeys by cholesterol feeding have failed⁴. The cholesterol added to the normal diets of these animals has not caused hypercholesteremia comparable to that occurring in rabbits.

A previous report from this laboratory⁵ showed that moderate hypercholesteremia (300 to 400 mg of cholesterol per hundred cubic centimeters of serum) maintained for forty-four to fifty-six weeks by feeding egg yolk powder to dogs did not give rise to arterial lesions. However, these levels were much lower than those attained in rabbits. In a recent article Li and Freeman⁶ reported that extreme hypercholesteremia, with cholesterol values as high as 1,700 mg per hundred cubic centimeters of serum, could be maintained in dogs for periods up to eighteen weeks by feeding cholesterol with a diet high in fat and deficient in protein. When the protein was adequate, the increases in serum

This investigation has been aided by a grant from the Albert and Mary Lasker Foundation.

From the Research Service, First (Columbia University) Division, Goldwater Memorial Hospital, Department of Hospitals, City of New York, and the Department of Medicine, College of Physicians and Surgeons, Columbia University.

1 Anitschkow, N. Beitr z path Anat u z allg Path **56** 379, 1913.

2 Dauber, D V, and Katz, L N. Arch Path **34** 937, 1942.

3 Bailey, C H. Proc Soc Exper Biol & Med **13** 60, 1915.

✓ 4 Anitschkow, N. Verhandl d deutsch path Gesellsch **20** 149, 1925.
Pfleiderer, E. Virchows Arch f path Anat **284** 154, 1932. Orto, L. ibid **269** 739, 1928.
Wacher, L, and Huech, W. Arch f exper Path u Pharmakol **74** 416, 1913.
Kawamura, R. Neue Beitrage zur Morphologie der Cholesterinsteatose, Jena, G Fischer, 1927.

5 Steiner, A, and Domanski, B. Am J M Sc **201** 820, 1941.

✓ 6 Li, T, and Freeman, S. Am J Physiol **145** 646, 1946.

cholesterol were only moderate. No atherosclerosis was observed in their dogs with high cholesterol levels.

It seems possible that in dogs and other omnivorous animals the mechanism for regulating cholesterol metabolism may continue to function effectively in spite of the ingestion of abnormal amounts of cholesterol. Production of hypercholesteremia may be dependent on our modifying this mechanism in some way. Many investigators have shown that the thyroid gland plays a part in the regulation of serum cholesterol levels.⁷ Unpublished experiments demonstrated that after thyroidectomy the addition of cholesterol to the diet resulted in marked hypercholesteremia in dogs.⁸ However, partial or complete removal of the parathyroid glands during the thyroidectomy made it difficult to maintain the dogs in a state of good nutrition.

In the investigation reported in this paper the thyroid activity of 4 dogs was modified by the administration of thiouracil⁹ and the effect on the serum cholesterol levels and on the development of arterial lesions was studied.

METHOD

Four adult mongrel dogs, 2 males and 2 females, weighing 20 to 53 pounds (9 to 24 kg), were employed. In the beginning of the experiment the dogs were fed approximately 325 Gm of a prepared food daily.¹⁰ Their appetite for this diet was erratic, therefore, after the first six months, the diet was changed to approximately 200 Gm of ground beef mixed with 125 Gm of the prepared food. The experiment was divided into three periods: an initial control period of eight weeks, a thiouracil feeding period of eight weeks and a thiouracil-plus-cholesterol feeding period of forty-eight to fifty-six weeks. The dogs were fed 0.5 Gm of thiouracil daily for the first month. This dose was increased to 0.8 Gm during the second and third months, to 1.0 Gm from the fourth through the ninth month and finally to 1.2 Gm from the tenth month until the end of the experiment. The amount of thiouracil was not increased beyond 1.0 Gm daily in dog 376. It was fed by placing 0.1 Gm tablets as far back in the posterior part of the pharynx as possible and then holding the jaws of the dog tightly closed. In the early phase of the experiment the dogs would occasionally regurgitate the thiouracil. This difficulty was overcome by allowing them to eat soon after the feeding of the drug. The thiouracil dose was increased three times in the first nine months because the elevated serum cholesterol levels gradually fell toward normal. The effect of thiouracil on the general bodily state was especially pro-

7 (a) Luden, G. *J. Lab. & Clin. Med.* **4**: 849, 1918. (b) Epstein, A. A., and Lande, H. *Arch. Int. Med.* **30**: 563, 1922. (c) Hurxthal, L. M. *ibid.* **51**: 22, 1933.

8 Steiner, A. Unpublished data.

9 Thiouracil was supplied by Dr. B. W. Carey, Lederle Laboratories, Inc., Pearl River, N. Y.

10 Spratt's Meat Fibrine Dog Cakes. The ingredients are wheat flour and meat meal, cooked. An analysis shows: crude protein, 20 per cent, crude fat, 2 per cent, fiber, 15 per cent, carbohydrates, 60 per cent, moisture, 7 per cent, ash, 2.5 per cent.

nounced during the last six to eight months of the study. This was evidenced by lethargy, inactivity, drowsiness and bouts of shivering. Basal metabolism determinations were not made.

Ten grams of crystalline cholesterol mixed in 40 cc of cottonseed oil was added daily to the diet of 3 of the 4 dogs (368, 377 and 378) after the first two months of thiouracil feeding. The fourth dog, 376, was maintained on thiouracil without the cholesterol supplement for the duration of this study and thus served as a control animal. The amount of the diet actually ingested varied considerably during the term of the experiment. It is estimated that throughout the course of the experiment the dogs ate from one half to two thirds of their diet. The dogs were weighed weekly. At the end of the experiment dog 368 had gained 7 pounds (3 Kg) while the others had lost weight: dog 378, 9 pounds (4 Kg), dog 376, 6 pounds (about 3 Kg), and dog 377, 5 pounds (a little over 2 Kg).

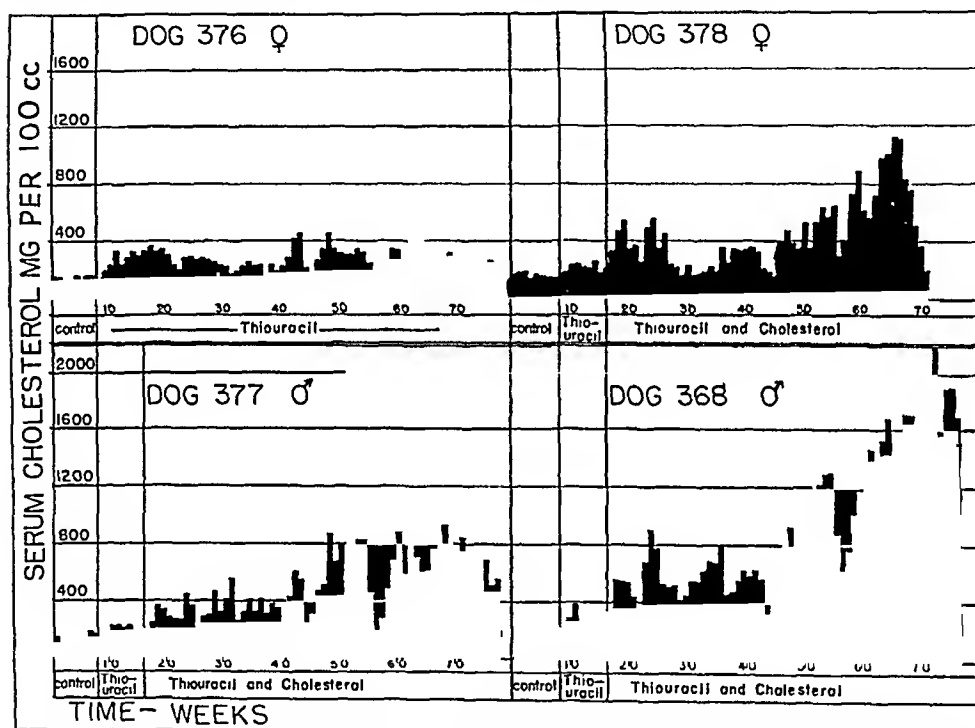


Fig 1—Levels of total serum cholesterol as determined at weekly intervals during the preliminary control period and during the periods when the dogs received thiouracil and thiouracil plus cholesterol

Because of the refusal of dog 378 to eat the ration, the addition of the cholesterol in oil was discontinued between the twenty-fourth and thirty-first weeks and between the thirty-ninth and forty-second weeks. The total cholesterol feeding period of this animal was twelve months, while with the other 2 dogs, 368 and 377, the period was fourteen months. Serum cholesterol determinations were made weekly by a modification of the Schoenheimer-Sperry method¹¹. Complete blood counts were made at bimonthly intervals.

At the end of the experiment the dogs were killed by exsanguination under pentobarbital sodium anesthesia.

¹¹ Schoenheimer, R., and Sperry, W. M. J Biol Chem 106 745, 1934

RESULTS

The serum cholesterol values of each dog are shown graphically in figure 1. The ranges of the serum cholesterol values for each period of the experiment, together with the average values, are summarized in the table. It can be seen that feeding thiouracil alone produced an increase of serum cholesterol. This was followed by a much greater increase when cholesterol in cottonseed oil was added to the diet. As can be seen from figure 1, the level fluctuated considerably from week to week. Abrupt decreases in serum cholesterol always followed a decrease in cholesterol intake. This is especially apparent in the two periods during which cholesterol was not fed to dog 378 and in the period following the fiftieth week when all the dogs ate poorly. During the experiment the ratio between free and esterified cholesterol in the serum remained unchanged, approximately 1 to 2, even when the total cholesterol content of the serum was at its highest.

Serum Cholesterol Values

| Dog | Control Period | | Thiouracil | | Thiouracil and Cholesterol | |
|--------------|------------------|--------|------------------|--------|----------------------------|--------|
| | Range, | Months | Range, | Months | Range, | Months |
| | Mg per 100 Cc | | Mg per 100 Cc | | Mg per 100 Cc | |
| 368 (male) | 141-191 (av 157) | 2 | 221-348 (av 308) | 2 | 403-2,176 (av 979) | 14 |
| 377 (male) | 108-186 (av 147) | 2 | 192-252 (av 216) | 2 | 196-932 (av 534) | 14 |
| 378 (female) | 139-192 (av 162) | 2 | 160-333 (av 208) | 4 | 208-1,134 (av 509) | 12 |
| 376 (female) | 136-158 (av 152) | 2 | 149-490 (av 284) | 16 | | |

The blood counts of the 4 dogs remained essentially unchanged. Aggranulocytosis did not occur.

The 4 animals were examined post mortem by Dr. Joseph Victor.

Dog 368 (27.7 Kg, male)—Gross changes were observed in the aorta and its branches and in the heart, the liver, the gallbladder and extrahepatic bile ducts, the kidneys, the thyroid gland, the stomach and the small intestine. Although the thoracic portion of the aorta was clear and smooth, elevated cream-colored and gray plaques appeared in the abdominal portion below the diaphragm and increased in size and number toward and beyond the bifurcation of the iliac arteries. The plaques were conspicuous about the orifices of the branches (fig. 2). Similar changes were evident in the coronary, pulmonary, thyroid, celiac, mesenteric, renal, iliac and femoral arteries. The lesions in the coronary arteries and their branches measured up to 2 cm in length and seemed to fill the lumens. The thyroid arteries were converted into straight, rigid, thickened vessels about 1.5 mm in diameter. Calcified plaques were present in both iliac arteries. No lesions were found in the cerebral, carotid, retinal or splenic arteries.

Although only moderate amounts of fat were seen in the subcutaneous, mesenteric, omental and retroperitoneal tissues, fatty deposits were prominent in the heart, the liver, the gallbladder, the bile ducts and the kidneys. The heart weighed 171 Gm and its muscle, especially that of the right ventricle, was mottled with yellow streaks. The liver weighed 813 Gm and was yellow and greasy. The gallbladder and the bile ducts had a velvety mucosa with glistening, bright



Fig 2—Abdominal aorta, iliac artery and femoral artery of dog 368 The aorta shows arteriosclerotic plaques at the orifices of branches, the iliac and femoral arteries show arteriosclerosis The dark plaques are calcified

yellow villi, which in places had coalesced to form bright golden nodules measuring up to 2 mm in diameter. The kidneys weighed 119 Gm and had dark brown cortices with "hairlike" yellow streaks in the intermediate zones. The adrenal glands weighed 21 Gm. Beginning in the cardia of the stomach and extending throughout the small intestine to the ileocecal valve, the muscularis was brown. The mucosa, in contrast, was creamy white. The colon was unaltered. The thyroid gland was greatly enlarged, the combined weight of the lobes being 9 Gm.

On microscopic examination, features characteristic of arteriosclerosis were apparent in the lesions of the abdominal portion of the aorta and in the coronary, iliac, femoral and thyroid arteries. In all these vessels the intima showed foam cell infiltration, which in many cases penetrated the inner part of the media. The internal elastica was thickened, reduplicated and occasionally fragmented. Elastic fibrils were seen in the thickened intima. Anisotropic cholesterol crystals were present within the foam cells. The abdominal portion of the aorta had, in addition, areas of intimal hyalinization (fig 3 A). The main coronary and thyroid arteries showed layers of fibrous connective tissue between the intimal foam cells (figs 3 B and 4 A). Several calcified intimal plaques which extended into the media were present in the internal iliac arteries.

Examination of organs showed atherosclerotic lesions in branches of the coronary, pancreatic (fig 4 B), renal, testicular and mesenteric arteries. These lesions were characterized by intimal collections of foam cells without fibrosis or hyalinization.

The pulmonary arteries had intimal changes which differed considerably from those of other arteries. These lesions were considered to be endarteritis obliterans.

Histologic changes were present in many other tissues. Myocardial fibrosis was found in small areas near the thickened coronary arteries. Excessive quantities of fat were observed in fat cells between myocardial fibers and within epithelial cells of the liver, Henle's loops of the kidneys, intrahepatic and extrahepatic bile ducts, the gallbladder and the mucosa of the small intestine. Ceroid pigment, which was brilliant red in Ziehl-Neelsen stains, occurred within histiocytes of the liver, the epididymis, lymph nodes, the spleen, the stomach and the small intestine as well as within smooth muscle cells of the cardia, the pylorus and the small intestine. In the distal convoluted tubules of the kidney there were great quantities of amorphous globules of brown pigment, which was orange-colored in Ziehl-Neelsen preparations. The thyroid gland was hypertrophied and hyperplastic, with papillary infoldings of the acini lined by cuboidal and columnar epithelium up to four cells thick. Colloid was often absent from the acinous spaces. The testes showed no spermatogenesis, the tubules being lined by swollen Sertoli cells. Many of the chromophobe cells of the anterior lobe of the pituitary gland had foamy cytoplasm, and some contained homogeneous eosinophilic spherical bodies about 15 to 20 microns in diameter.

Dog 377 (91 Kg, male)—Gross changes were seen in the abdominal portion of the aorta and its branches and in the heart, the liver, the gallbladder, the bile ducts, the kidneys, the thyroid gland and the small intestine. Cream-colored intimal plaques were found in the aorta, beginning at the diaphragm and increasing in number and size peripherally. Similar lesions were also observed in the coronary, celiac, renal, mesenteric, iliac and thyroid arteries. The lesions were less severe than those in dog 368. No calcification was noted. Fatty changes were seen in the heart and the liver. The heart weighed 104 Gm, and there was yellow mottling of the myocardium. The liver weighed 478 Gm and was yellow, fat and firm. The gallbladder and the bile ducts had a velvety mucosa,

which was tinted with glistening golden-tipped villi. The kidneys weighed 54 Gm and had dark brown cortices. The adrenal glands weighed 18 Gm and had wide cortical zones. The jejunum had tan-colored muscularis, while the mucosa of the entire small intestine was creamy white. The thyroid gland weighed 8.5 Gm.

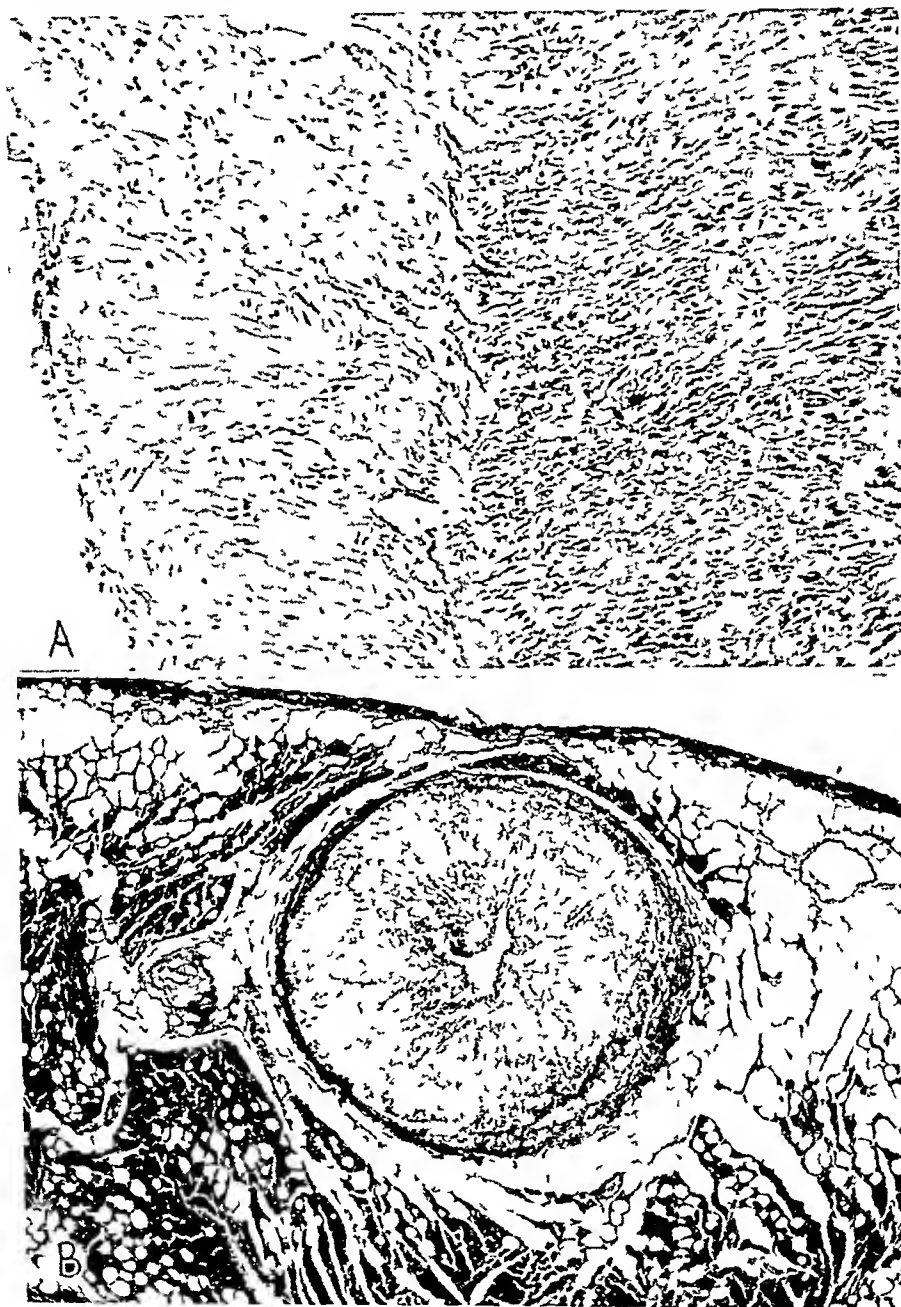


Fig 3—*A*, photomicrograph of abdominal aorta. The intimal plaque consists of hyalinized connective tissue, lymphoid cells and mononuclear cells with foamy cytoplasm. The mononuclear cells also occur in the inner part of the media. Giemsa stain $\times 100$.

B, photomicrograph of the coronary artery and myocardium. The intima of the artery is thickened by fibrous connective tissue and foam cells. Most of the media is replaced by these cells. Recanalization of the plaque has occurred. Many fat cells separate the myocardial fibers. Masson's trichrome stain $\times 50$.

Microscopically, the arterial lesions consisted of intimal deposits of fat and infiltrating foam cells without hyalinization or fibrosis. The foam cell infiltration penetrated the inner part of the media in many lesions. Changes in the smaller branches of the arteries were seen only in the thyroid gland. Fatty deposits were present in the organs. The fatty changes were similar in character and

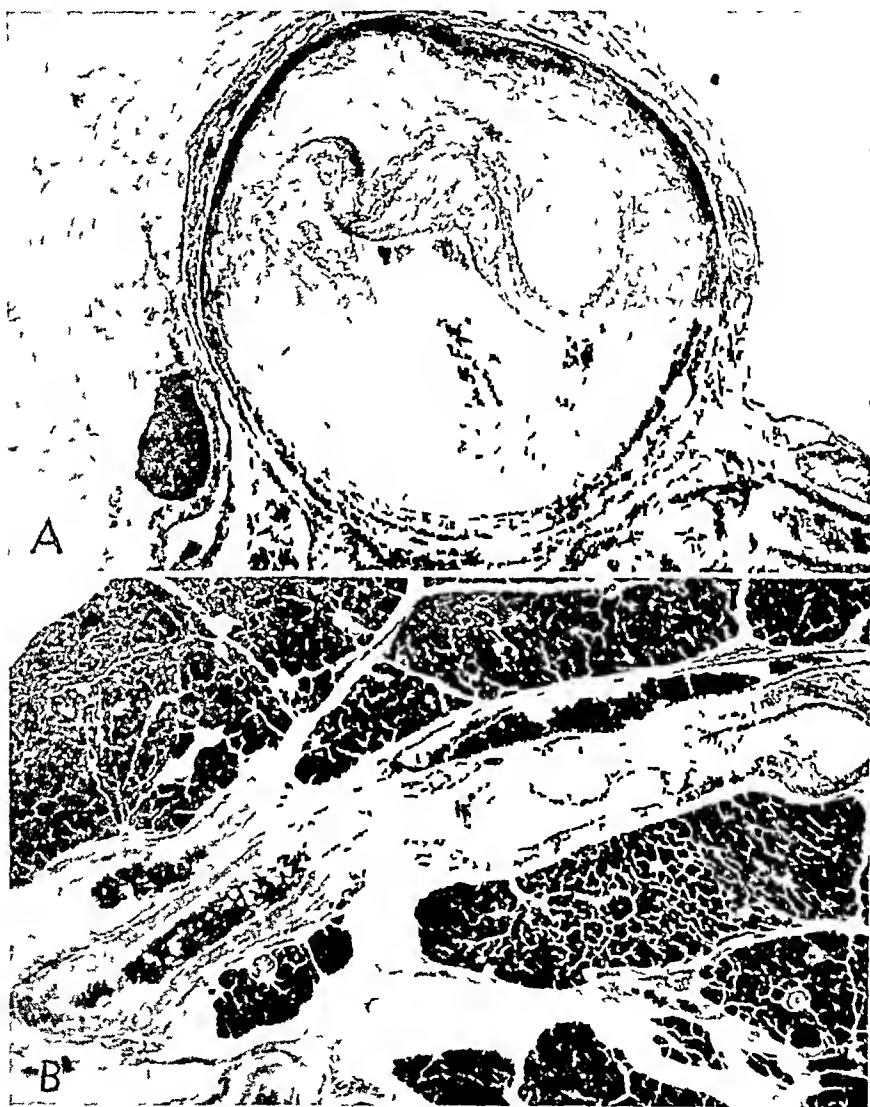


Fig 4—*A*, photomicrograph of the thyroid artery. Note the intimal fibrosis and foam cell infiltration. The media is occupied by foam cells and fat spaces. Masson's trichrome stain $\times 50$.

B, photomicrograph of the pancreatic artery. The thickening of the intima is due to foam cells. Masson's trichrome stain $\times 50$.

distribution to those described for dog 368 but were less extensive. Ceroid was found in the smooth muscle cells and histiocytes of the jejunum. Excessive quantities of brown pigment which was not acid fast occurred in the renal distal convoluted tubules. Hyperplasia of the thyroid gland resembled that seen in dog 368. Spermatogenesis was active.

Dog 378 (9.5 Kg, female)—There were changes in the thyroid gland, the liver, the gallbladder and the kidneys. The aorta and its large branches were unaltered. The hypertrophy of the thyroid gland was extraordinary, the combined weight of the two lobes being 130 Gm. The liver weighed 490 Gm. It was yellow, fat and greasy and had a finely granular capsule. The mucosa of the gallbladder was velvety with glistening golden yellow villi, many of which coalesced to form xanthomatous masses measuring up to 3 mm in diameter. The extrahepatic bile ducts had mucosa resembling that of the gallbladder. The renal cortices were dark brown with scattered gray flecks about 0.5 mm in diameter. Hairlike yellow streaks were present in the intermediate zones. Organ weights in grams were: heart, 640, spleen, 260, kidneys, 450, adrenal glands, 20.

Microscopic evidence of vascular lesions was found only in the arteries of the thyroid gland and the ovaries. The essential change was atherosclerosis with subendothelial deposits of enormous foam cells. In the ovarian arteries the atherosclerosis was associated with thrombosis caused by blood elements and recanalization by proliferating endothelium-lined capillaries. Other noteworthy tissue alterations were present. Extraordinary deposits of fat were found in the epithelial cells of the liver, the intrahepatic bile ducts, the gallbladder, the renal loops of Henle and between the cardiac muscle fibers. Intense pigmentation with a brown amorphous granular material occurred in the epithelial cells of the renal distal convoluted tubules. The pigment had an orange color with Ziehl-Neelsen stain. Large masses of acid-fast ceroid pigment occurred in the histiocytes of the liver, the ovarian stroma, the uterine endometrium, the hypophyseal pars nervosa and the lymph nodes. Thyroid hyperplasia and hypertrophy were extreme. Colloid was often absent from the acinous spaces. The anterior lobe of the pituitary gland had many swollen cells about three times the usual diameter, with ground glass, foamy or partly hyalinized cytoplasm.

Dog 376 Thiouracil Control (11.4 Kg, female)—The only changes were enlargement of the thyroid gland and brown pigmentation of the renal cortex and the muscle of the jejunum. The aorta and its branches were unaltered. On microscopic examination hypertrophy and hyperplasia of the thyroid gland were apparent. A pigment having the characteristics of ceroid was present in the jejunal muscle fibers and in the histiocytes between the longitudinal and circular layers of muscle. Similar pigment occurred in a few histiocytes of ovarian and endometrial stroma. The brown color of the renal cortex was due to the presence of small amounts of a pigment similar to that found in the other dogs. The blood vessels were normal. Weights of organs in grams follow: heart, 98, spleen, 206, liver, 280, adrenal glands, 15, kidneys, 351, thyroid gland, 51.

COMMENT

Hypercholesteremia was produced in dogs and maintained for long periods by feeding cholesterol after the function of the thyroid gland had been modified by administration of thiouracil. A daily dose of from 0.5 to 1.2 Gm of thiouracil without cholesterol produced increases of from 50 to 140 mg per hundred cubic centimeters in the average serum cholesterol content. This increase is of the same magnitude as that which followed thyroidectomy^{7c}. The addition of 10 Gm of cholesterol and 40 cc of cottonseed oil to the daily diet of 3 dogs receiving thiouracil resulted in extreme hypercholesteremia. The ele-

vation of level over the base line reached a maximum of 770, 980 and 2,000 mg per hundred cubic centimeters in the 3 dogs. Maintenance of the high level of serum cholesterol depended on both the cholesterol intake and the dose of thiouracil. Whenever the dogs ate poorly, thus reducing the cholesterol intake, the serum levels fell. The dose of thiouracil was increased at three different times during the experiment. In general, on these occasions further increases of the cholesteremia occurred in the cholesterol-fed dogs but did not occur in the control dog receiving only thiouracil.

Arterial lesions were produced in the 3 dogs receiving cholesterol. These lesions were considered to be those of atherosclerosis when the arterial intima showed foam cell infiltration without fibrosis, hyalinization or calcification. They were considered to be those of arteriosclerosis when one or more of these features were present together with foam cell infiltration. The extent and the severity of the arterial lesions paralleled the duration and the degree of the hypercholesteremia in these dogs. Dog 376, whose serum cholesterol level never exceeded 500 mg per hundred cubic centimeters, had no vascular lesions. Dog 378, whose serum cholesterol level was over 500 mg per hundred cubic centimeters for ten weeks, had atherosclerotic lesions in the ovarian and thyroid arteries only. Dog 377, with levels in excess of 500 mg per hundred cubic centimeters for twenty-seven weeks, had atherosclerosis of the abdominal portion of the aorta and its branches and of the coronary and thyroid arteries. Dog 368 had levels exceeding 500 mg per hundred cubic centimeters for fifty-two weeks and exceeding 1,000 mg for twenty-three weeks, in this animal atherosclerotic and arteriosclerotic lesions were widely distributed. The 3 dogs receiving cholesterol had abnormal fat deposits in the heart, the liver, the kidneys and the adrenal glands.

Hyperplasia and hypertrophy of the thyroid gland were seen in all the dogs. The changes resembled those described as resulting from thiouracil administration in other species¹². As might be expected the changes were more extreme in the thyroid glands of the smaller dogs since the dose of thiouracil was not adjusted to body weight. Thus dogs 377 and 378, weighing 11.4 Kg and 9.5 Kg, and control dog 376, weighing 11.4 Kg, showed much more thyroid hypertrophy than did dog 368, which weighed 27.7 Kg.

Ceroid pigment was observed in certain tissues of the 4 dogs fed thiouracil. Since the extent and the severity of the pigmentation were greatest in dog 368 and least in control dog 376, the phenomenon paralleled the hypercholesteremia rather than the hyperplasia of the thyroid gland. The distribution of the pigment, which was most abun-

¹² MacKenzie C. G. and MacKenzie J. B. *Endocrinology* **32** 185, 1943

dant in smooth muscle cells of the small intestine, resembled that observed in human beings with sprue¹³ and in dogs with bile fistula¹⁴. Although ceroid pigmentation may be influenced by dietary vitamin E,¹⁵ no evidence of vitamin E deficiency was found.

Progress in the study of arteriosclerosis is largely dependent on the ability to reproduce the disease in experimental animals. Because of the nature of the lesions, it is difficult to follow the development of the disease in human beings. Up to the present time lesions similar to those seen in human arteriosclerosis have been produced most successfully in the rabbit. In this species hypercholesteremia produced by feeding cholesterol is consistently followed by the development of arterial lesions. Although, in many respects these lesions resemble those seen in man,¹⁶ Duff and other investigators¹⁷ have emphasized certain differences. The distribution of the lesions differs from that most frequently seen in human atherosclerosis and arteriosclerosis. In rabbits the lesions are most concentrated in the arch and the thoracic portion of the aorta. In man the lesions occur mainly in the abdominal portion of the aorta and its branches. Inasmuch as similar lesions have not previously been reported to have been produced in omnivorous mammals such as the dog, the cat or the monkey, the suggestion has been made that the lesions in the rabbit represent the response of a herbivorous animal to a substance not normally present in its diet and that they have no relationship to the lesions occurring in man.

However, arterial lesions have now been shown to follow sustained hypercholesteremia in dogs. If the differences in the normal serum cholesterol levels and in the life spans of the two species are taken into consideration, the conditions necessary for the production of the arterial lesions are similar in rabbits and dogs. Although in the 2 dogs in which gross arterial lesions developed the serum cholesterol level was higher than that required to produce lesions in rabbits, the percentage increase in level was similar. Lesions appear in rabbits after the serum cholesterol has been maintained at a level from three to ten times the normal for a period of from six to ten weeks. Gross lesions were found in the dogs after the serum cholesterol had been maintained at levels from three to ten times the normal for dogs for at least twenty-seven weeks.

13 Pappenheimer, A. M., and Victor, J. *Am J Path* **22** 395, 1946.

14 Whipple, G. H., and Hooper, C. W. *Am J Physiol* **43** 275, 1917.

15 Martin, A. J. P., and Moore, T. *Chem & Indust* **57** 973, 1938.

16 Anitschkow, N., in Cowdry, E. V. *Arteriosclerosis*, New York, The Macmillan Company, 1933. Leary, T. *Arch Path* **21** 459, 1936, **32** 507, 1941.

17 Duff, L. *Arch Path* **20** 81 and 259, 1935. Aschoff, L. *Brit M J* **2** 1131, 1932.

It is possible that the thyroid involvement in these dogs influenced the development of the arterial lesions in some way not connected with the hypercholesteremia. However, since no arterial lesions were found in the control dog, which had similar thyroid involvement without marked hypercholesteremia, the cholesterol level seems to be the decisive factor.

The distribution of the arterial lesions in these dogs is different from that observed in rabbits and is strikingly similar to that found in human arteriosclerosis. In morphologic characteristics the lesions vary from atherosclerotic lesions similar to those seen in rabbits to lesions with fibrosis, hyalinization and calcification similar to those seen in human material.

It is probable that the fundamental disease process is the same in dogs and rabbits and that the differences in the distribution and the morphologic character of the arterial lesions are due to some unidentified anatomic factor. The ability of the dog to metabolize considerable quantities of cholesterol increased the difficulty of maintaining a condition of hypercholesteremia but did not prevent the development of arterial lesions after the hypercholesteremia was established.

Although arteriosclerosis of man is too protean a disease to have a single cause, the results reported here justify the belief that at least in the cases in which arteriosclerosis follows hypercholesteremia the disease is similar to that produced experimentally in animals. It seems important, therefore, to continue studies of factors which may modify the lesions following hypercholesteremia in experimental animals.

SUMMARY

Arterial lesions similar in distribution and morphologic character to those seen in human atherosclerosis and arteriosclerosis were found in dogs following prolonged hypercholesteremia. The high serum cholesterol levels were produced by feeding 10 Gm. of cholesterol in 40 cc. of cottonseed oil daily to dogs whose thyroid function was modified by administration of thiouracil.

FURTHER EVIDENCE FOR SUCCESSIVE STAGES IN THE FORMATION OF NEOPLASMS

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ONE OF the most significant findings in the field of cancer research is the observation that carcinogenesis can be augmented or retarded by certain extrinsic or intrinsic agents¹. In this field investigations have a far greater importance than the mere search for agents with cocarcinogenic or anticarcinogenic properties, since such studies have provided some fundamental concepts about carcinogenesis itself. While the work is incomplete, two corollary conclusions concerning this process appear justified at this time. First, cancer formation occurs not as a continuous single process but rather as a series of biologic changes; second, certain forces other than those actually involved in the genesis of the tumor cell are necessary for the formation of grossly perceptible neoplasms.

Although these two conclusions have been expressed previously,² there is no unanimity of opinion as to the exact role of the various forces necessary for tumor formation or as to the sequence of their action. One theory holds that the carcinogen first induces a neoplastic cell or cells from normal tissue and then stimulates the proliferation of such cells. This proposal emphasizes the difference in the genesis and the growth of neoplasms and in various forms it has received the support of Berenblum,^{1a} Kline and Rusch^{2a} and Friedewald and Rous.^{1b} A second hypothesis, advanced by Simpson and Cramer,³ proposes that methylcholanthrene is a sensitizing agent, itself noncarcinogenic, that prepares the cells for subsequent carcinogenic action by the metabolic

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This investigation was aided by the Jonathan Bowman Fund for Cancer Research.

1 (a) Berenblum, I. *Arch Path* **38** 233, 1944. (b) Friedewald, W F, and Rous, P. *J Exper Med* **80** 101, 1944. (c) Kreyberg, L. *Acta path et microbiol Scandinau* (supp) **37** 317, 1938. (d) Rusch, H P. *Physiol Rev* **24** 177, 1944.

2 (a) Kline, B E, and Rusch, H P. *Cancer Research* **4** 762, 1944. (b) MacKenzie, I, and Rous, P. *J Exper Med* **73** 391, 1941. (c) Rous, P, and Kidd, J G. *ibid* **73** 365, 1941. (d) Tannenbaum, A. *Cancer Research* **4** 678, 1944. (e) Berenblum^{1a}. (f) Friedewald and Rous^{1b}. (g) Rusch^{1d}.

3 Simpson, W L, and Cramer, W. *Cancer Research* **5** 5, 1945.

The Influence of Wool Fat and Croton Oil on Carcinogenesis Caused by Methylcholanthrene in Mice

| Cumulative Tumor Count at Given Number of Months After the First Application of the Carcinogen | | | | | | | | | | | | | | | | | | | Num ber of Mice Tumor Free | | |
|---|--|---|--------------------------|---|----|----|---|----|---|----|---|----|----|-----|----|---|----|----|--|----|---|
| Group | Treatment * | | Effec tual Total † | 7 | | | | | | | | | | | | | | | 9 | | |
| | First Period, 4 Months | Second Period, 3 Months | | 3 | | | 4 | | | 5 | | | 6 | | | Per centage of Mice with Tumors ‡ | P | Ca | Percentage and of Mice Alive with at Nine Months † | | |
| | | | | P | Ca | § | P | Ca | P | Ca | P | Ca | P | Ca | P | | | | | Ca | P |
| 1 | Methylcholanthrene in benzyl alcohol | Same as first period | 38 | 2 | 0 | 15 | 0 | 29 | 0 | 27 | 8 | 16 | 22 | 100 | 12 | 26 | 4 | 34 | 100 | 0 | |
| 2 | Methylcholanthrene in wool fat | Same as first period | 40 | 0 | 0 | 0 | 0 | 2 | 0 | 3 | 0 | 9 | 1 | 25 | 14 | 5 | 18 | 7 | 62 | 12 | |
| 3 | Methylcholanthrene in wool fat | Methylcholanthrene in benzyl alcohol | 40 | 0 | 0 | 0 | 0 | 4 | 0 | 18 | 0 | 28 | 9 | 92 | 20 | 18 | 14 | 24 | 95 | 0 | |
| 4 | Methylcholanthrene in wool fat | Croton oil in benzyl alcohol | 40 | 0 | 0 | 1 | 0 | 7 | 0 | 13 | 2 | 22 | 3 | 62 | 21 | 7 | 19 | 7 | 64 | 7 | |
| 5 | Methylcholanthrene + croton oil in wool fat | Same as first period | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 10 | 0 | 25 | 15 | 3 | 18 | 7 | 62 | 14 | |
| 6 | Methylcholanthrene in benzyl alcohol | Methylcholanthrene in wool fat | 34 | 3 | 0 | 18 | 0 | 26 | 1 | 22 | 7 | 18 | 14 | 94 | 11 | 17 | 6 | 20 | 77 | 0 | |

* Methylcholanthrene was used as a 0.3 per cent and croton oil as a 1 per cent solution in all cases

† This total was the number of animals alive at the time the first tumor appeared

‡ P stands for papilloma, Ca, for carcinoma

‡ Percentage of mice with tumors is based on the effectual total Both those with papilloma and those with carcinoma are included

derivatives of the hydrocarbon Both theories are based on evidence that carcinogenic hydrocarbons possess biologic properties other than those involved in the genesis of the tumor cells The experiments reported in the following pages were designed to obtain further information concerning these various properties and to present additional evidence that supports the existence of well defined stages in the development of neoplasms

MATERIALS AND PROCEDURE

A series of 240 ABC young adult albino mice of both sexes were divided into six groups of 40 each The sexes were separated and were kept in groups of 20 in metal box cages on shavings, with dog biscuit and water available at all times The experiment was divided into two periods The initial course of treatment was continued for four months and was followed by a second of three months' duration For the second period the treatment was changed in some of the groups Initially the mice were painted twice weekly with a solution of 0.3 per cent methylcholanthrene in benzyl alcohol, in wool fat (anhydrous) or in wool fat containing 1 per cent croton oil The various preparations were applied to the skin of the interscapular area with a camel's hair brush Because of the pasty character of the mixtures that contained wool fat there was some doubt as to whether the same amount of methylcholanthrene had contact with the skin as when benzyl alcohol was the solvent To obviate this difficulty, the hair was clipped from the painted area every two weeks In the second period, one of the groups (no 4) was painted with a cocarcinogenic mixture consisting of 1 per cent croton oil in benzyl alcohol, and another group (no 5) received a mixture of 0.3 per cent methylcholanthrene and 1 per cent croton oil in wool fat The remaining groups continued to receive methylcholanthrene, either in benzyl alcohol or in wool fat The exact treatment given in both periods is shown with the results in the table The mice were examined carefully at weekly intervals for the appearance of tumors and were kept an additional two months beyond the last application of the carcinogen in order that latent effects might be observed The growth of the neoplasms was closely followed, and mice were removed from the experiment only after the tumors had attained a size sufficient to impair their health

RESULTS

The rate at which tumors appeared in the various groups is shown in the table Since no striking difference in the incidence of tumors in the sexes was found, the numbers observed in the two sexes are not listed separately When a solution of methylcholanthrene in benzyl alcohol was used, tumor formation was rapid, and at the end of the fourth month tumors (papilloma) were observed in 15 mice in group 1, and all of the mice in this group had tumors after the seventh month In striking contrast to these results were those obtained when the carcinogen was dissolved in wool fat, no mice had tumors at four months and only 10 had them at seven months, but the incidence slowly increased to 62 per cent during the next two months (group 2) This is a higher incidence than that reported by Simpson and Cramer,³ but the mice in the present series received a greater total amount of

carcinogen over a longer period. Besides the decrease in incidence of the tumors it is also of interest that only 7 mice with carcinoma were found in the group that received the carcinogen in wool fat, in contrast to 34 in the control group. It is obvious from this experiment that wool fat greatly retards but does not completely prevent tumor formation.

In order to obtain further information concerning the mode of action of wool fat and of methylcholanthrene, the latter was applied in different solvents at various periods. In one group the carcinogen in wool fat was painted on the skin the first four months, followed by a three month period during which the hydrocarbon was applied in a solution of benzyl alcohol. In none of the mice in this group did perceptible tumors develop during the initial four months of treatment, but neoplasms occurred rapidly in this group soon after the hydrocarbon-benzyl alcohol mixture was used, and by the end of the second period 92 per cent of the mice had tumors (group 3). There are at least two explanations for such results. Methylcholanthrene might have induced the formation of tumor cells following its application in wool fat but failed to stimulate such cells to proliferate because of some "neutralizing" or inhibiting effect of the wool fat. The growth of such "tumor rests" could then occur following the subsequent application of the carcinogen in the absence of wool fat. A second explanation is that proposed by Simpson and Cramer³, i.e., wool fat retarded the breakdown of methylcholanthrene, and the unaltered hydrocarbon acted merely as a sensitizing agent during the first period, thus preparing the skin for subsequent action of the metabolic derivatives of the carcinogen. If the former explanation is correct, it should be possible to evoke tumors after the initial carcinogenic treatment by the application of a non-carcinogenic irritant such as croton oil, whereas such a nonspecific irritant would not be expected to call forth tumors if the suggestion of Simpson and Cramer is valid. Group 4 was designed to test this point and differed from group 3 only in that a 1 per cent solution of croton oil was substituted for the methylcholanthrene in the second period. The influence of croton oil was similar to, but not as pronounced as, that obtained with the hydrocarbon. Tumor formation was accelerated following the application of the irritant and at seven months 62 per cent of the mice had tumors (group 4) compared with an incidence of 25 per cent in group 2, in which the mice had received methylcholanthrene dissolved in wool fat for the entire seven months. The cocarcinogenic effect of croton oil was largely confined to the period during which it was applied, and few new tumors were evoked after the cessation of treatments at the end of seven months. Thus the incidence of neoplasms rose to 70 per cent at the end of eight months, but during the next month the tumors (papilloma) in 2 mice regressed,

giving a final tumor incidence of 64 per cent at the end of the experiment Methylcholanthrene, on the other hand, not only stimulated the formation of neoplasms already present but was also responsible for the genesis of new tumors when it was applied during the second period (group 3)

It is possible that the anticarcinogenic effect of wool fat is due to its ability to neutralize the "irritating" influence of methylcholanthrene and croton oil In order to test this idea the mice of group 5 were painted for seven months with a solution of wool fat containing both methylcholanthrene and croton oil In the absence of wool fat this combination of carcinogen and cocarcinogen is known to accelerate the induction of tumors⁴ The cocarcinogenic influence of croton oil was completely nullified in this experiment, and the results are perfect checks of those obtained in group 2, in which croton oil was absent The inhibiting effect of wool fat may also be due to its physical characteristics rather than to any more subtle neutralizing powers Its pasty consistency might permit only small amounts of the dissolved hydrocarbon to obtain contact with the surface of the skin and this in effect would result in the application of subcarcinogenic doses, which are known to induce similar changes^{2a}

To determine whether wool fat can inhibit the neoplastic process, once this has been initiated, a sixth group of mice were painted for the first four months with a solution of methylcholanthrene in benzyl alcohol, after which the hydrocarbon was applied to the skin in a solution of wool fat Eighteen mice of this group had tumors before application of the wool fat-hydrocarbon mixture was started, and tumors continued to form rapidly thereafter even in the presence of the wool fat, 94 per cent of the mice had tumors at the end of seven months However, the percentage of mice with cancers was slightly less than was found in the group that received the carcinogen in the absence of wool fat (group 1) Furthermore, a considerable number of tumors (papilloma) in group 6 regressed during the last two months of the experiment, and at the end of the ninth month the incidence of tumors was reduced to 77 per cent It is possible that the initial period of treatment with the carcinogen was too long, and a greater differential between the results of group 6 and its control (group 1) could have been demonstrated if the application of wool fat had been started at the end of the third month At all events it appears that wool fat is capable of neutralizing or inhibiting the properties of either carcinogen or cocarcinogen that are responsible for the proliferation of neoplastic tissue

COMMENT

Although the carcinogenic process has never been regarded as a simple one, recent investigations have indicated even greater complexities

4 Bereblum, I (a) *Cancer Research* **1** 44, 1941, (b) **1** 807, 1941

than were previously suspected. Cancer formation appears not to occur as a continuous single process but rather as a series of biologic changes. Furthermore, there are definite indications that carcinogenic hydrocarbons are active only because of several distinct biologic properties and neoplasms seem to develop only as the result of the proper sequential summation of these properties. That more than one quality of a carcinogen is involved in the formation of tumors gains support from the fact that some characteristics of hydrocarbons may be masked and others simulated by certain compounds. For example, the observation that an inhibiting effect is exerted on the carcinogenic activity of certain hydrocarbons by wool fat and by liquid petrolatum has been well established.⁵ These anticarcinogens appear to neutralize or to mask the irritating effect of the hydrocarbons without affecting their ability to induce neoplastic cells since noncarcinogenic chemical or physical irritants quickly call forth neoplasms in tumor-free skin which had previously been treated with the hydrocarbons dissolved in wool fat or liquid petrolatum.⁵ When wool fat was used as a solvent for methylcholanthrene, it largely abolished the irritating effects of the hydrocarbon on the skin, and epilation, destruction of sebaceous glands and epidermal hyperplasia were all minimal.³ Such data suggest at least two biologic properties of carcinogenic hydrocarbons which are essential for the development of tumors: the ability to induce neoplastic cells and the ability to stimulate these cells to proliferative activity. The factor responsible for the genesis of the tumor is relatively bland and has not been duplicated to our knowledge with noncarcinogenic substitutes, while the property that causes cellular multiplication is due to a nonspecific irritant of the carcinogen and can be reproduced by other agents.⁶

The present experiments provide further evidence in favor of the concept that cancer formation results from a sequence of biologic changes. As a working hypothesis, the three periods listed in an earlier communication^{2a} will be considered in light of the more recent findings.

1 *Period of Induction*—This stage has also been referred to as the period of preparation, latency, initiation or preneoplasia.⁷ Since some arbitrary limitations must be defined, this phase should include only the period of genesis of the neoplastic cell or cells from normal tissue, and it should not be confused with the multiplication of these cells.

Although any concept of carcinogenesis must include a period covering the genesis of the neoplastic cell, the exact sequence of this event in the process has not been demonstrated. Certainly it occurs

5 Friedewald and Rous^{1b} Simpson and Cramer³

6 (a) Pullinger, B. D. J. Path. & Bact. **55** 301, 1943 (b) Berenblum^{1a}
(c) Friedewald and Rous^{1b} (d) Kline and Rusch^{2a} (e) Rusch^{1d}

7 Berenblum^{4b} Tannenbaum^{2d}

early, but in certain types of cancer it may be preceded by a period of preneoplasia during which tissues become biased toward forming neoplastic cells. In certain circumstances such a preliminary phase seems to be essential for the subsequent genesis of tumors. For example, certain cells in atrophied mammary tissue in male mice of a high cancer strain may have the potentialities for tumor development, but few if any tumors occur unless the glandular epithelium is first prepared by estrone.⁸ Huseby and Bittner⁹ have shown that precancerous nodules appear in mammary tissue prior to cancer formation, and other pre-neoplastic lesions have been long recognized. It is assumed that the opportunities for neoplastic transformation of normal tissue have been increased considerably during this preliminary period. Thus a "proper soil" appears essential for the genesis of some neoplasms, but there is no reason to believe that such preparation is universally required for all types of tumors. The observation of Mottram¹⁰ that croton oil accelerates tumor formation when applied to the epidermis prior to the application of a carcinogen is contrary to most findings and must be confirmed, and although Simpson and Cramer³ postulate sensitization as a prerequisite to the genesis of the neoplastic cell, our results indicate that stimulation follows the formation of the tumor cell. Nevertheless, the available evidence, although not conclusive, is sufficient to justify the subdivision of the period of induction of certain tumors into two phases: (a) stage of preneoplasia and (b) stage of genesis.

2 Critical Period or Period of Reversibility—This starts at the moment at which the genesis of the neoplastic cell has been completed and is that stage in which cellular proliferation is delicately balanced. During this phase there are so few neoplastic cells present that they are more or less lost among the normal cells, and since their mass has not yet attained a size sufficient to receive a direct supply of blood, they must compete with the healthy cells for the nutrients in the fluids of the tissue spaces. At such times these cells are the most susceptible to the influence of their environment, presumably during this phase some tumor cells proliferate, some lie quiescent for varying periods and others succumb. Small nests of tumor cells have less chance of survival than larger clusters,¹¹ and the fate of small masses of neoplastic tissue depends on the balance between their proliferative capacity and the amount of encouragement or of resistance present in the neighboring tissues. This period could vary from a few hours to several weeks or months.

⁸ Gardner, W. U. *Arch. Path.* **27**, 138, 1939.

⁹ Huseby, R. A., and Bittner, J. J. *Cancer Research* **6**, 240, 1946.

¹⁰ Mottram, J. C. *J. Path. & Bact.* **56**, 391, 1944.

¹¹ Reinhard, M. C., Goltz, H. L., and Warner, S. G. *Cancer Research* **5**, 102, 1945.

The concept of a critical period in the development of a tumor is supported by several types of experimental evidence,^{2a} but of special consequence is the evidence of a latent period under certain conditions of carcinogenesis. When a carcinogenic hydrocarbon is applied to the skin of mice for a time short of that necessary to induce tumors, the subsequent resumption of the hydrocarbon treatment will quickly precipitate the formation of tumors even though three to four months intervene between the two periods of application of the hydrocarbon.^{2a} Actually in certain cases a noncarcinogenic agent, such as heat, a wound or croton oil, may substitute for the hydrocarbon in the second period.¹² Such observations indicate that the initial application of the hydrocarbon produced alterations in the tissues that persisted for at least four months, and we may assume either dormancy of a partially completed chemical reaction which is essential to carcinogenesis and which may be reinitiated and completed by a second application of the carcinogen at some subsequent date, or we may consider that one phase of the carcinogenic reaction, i e, the genesis of tumor cells, had been completed and such cells remained quiescent until stimulated to proliferation at some later date. Since the concept of an interrupted chemical reaction does not appear too plausible, it seems more likely that the latter suggestion is the more correct. At all events, it is known that tumor cells may persist in a quiescent state for as long as sixteen to twenty-nine weeks before perceptible growth is observed.¹³

The importance of factors that are responsible for the realization of tumors has been mentioned by several authors,¹⁴ but it is our contention that the influence of these forces is most pronounced during the critical period. Gardner^{13c} observed that testicular interstitial cell "tumor rests" could be stimulated to proliferation following the injection of estrogens, and the cocarcinogenic influence of most chemical and physical irritants is also most effective when applied subsequent to a period of carcinogenic treatment.¹⁵ It is also of interest that high calory diets have relatively little stimulative effect during the period when cancer is being initiated or on the growth of established tumors but are definitely stimulative if given just after the preliminary period of carcinogenic treatment, i e, the critical period.¹⁶

12 Berenblum^{1a} Friedewald and Rous^{1b} Kline and Rusch^{2a} Ellinger^{6a}

13 (a) Ellinger, F. *Nature*, London **142** 151, 1938 (b) Fischer, A. *Am J Cancer* **31** 1, 1937 (c) Gardner, W. U. *Cancer Research* **5** 497, 1945

14 Fischer^{13b} Friedewald and Rous^{1b} Kline and Rusch^{2a} Tannenbaum^{2d}

15 Cramer, W. *Brit J Exper Path* **10** 335, 1929 Berenblum^{1a} Friedewald and Rous^{1b} Rusch^{1d}

16 Rusch, H. P., Kline, B. E., and Baumann, C. A. *Cancer Research* **5** 431, 1945

The length of the critical period, no doubt, varies for different tumors. Neurofibroma developing in the ear of the rat as a result of prolonged feeding of crude ergot regressed when such feeding was discontinued but recurred when the administration of ergot was resumed¹⁷. This type of tumor cell failed to proliferate in the absence of certain stimulating factors, and the tumor never became autonomous. The anterior chamber of the eye has been shown to be an excellent location for the growth of many types of tumors, and it illustrates a region of favorable environment for the proliferation of tumor cells during the critical period¹⁸. Once the neoplasm becomes autonomous, its cells may proliferate in a variety of tissues.

3 Period of Progression—This may be considered as that period when the neoplastic cells have gained ascendancy over the forces that hold them in control. The tumor has established its own vascular supply and no longer needs to compete with normal cells for the nutrients in the tissue spaces^{2a} but in general is in a period of relatively unrestricted, invasive growth, during which regressions are infrequent. The exact size that a neoplasm must attain before it reaches this stage varies, no doubt, with different tumors, but all grossly perceptible tumors are probably in this phase of their growth. Although there is no sharp line between the second and the third period, there is some evidence in favor of such distinction. As stated previously, Reinhard, Goltz and Warner¹¹ have shown that the chance of survival of a transplantable mouse adenocarcinoma was proportional to the number of cells inoculated and that the latent period for a tumor "take" increased as the number of injected cells decreased. They have further observed more regressing tumors in animals inoculated with dilute suspensions, while none were seen in mice inoculated with concentrated suspensions. If our point of view is correct, it suggests that all inoculated tumor cells, like spontaneous neoplasms, pass through a critical period before they become established. It seems probable that spontaneous tumor cells face a similar but, most often, more precarious critical period.

The proposed three periods represent only an arbitrary scheme intended to assist in the clarification of our views on carcinogenesis and to facilitate further experimentation. Possibly the different stages are not clearly separable, and within a neoplastic focus at any period tumor cells are being formed, others are dividing and still others are dying. The final outcome is the result of a balance of the various factors involved. At all events, the ability of tumor cells to remain dormant for long periods and to respond to noncarcinogenic stimulation by multiplying

17 Nelson, A. A., Fitzhugh, O. G., Norris, H. J., and Calverly, H. O. *Cancer Research* 2: 11, 1942.

18 Greene, H. S. N., in *Research Conference on Cancer*, American Association for the Advancement of Science, 1945, p. 154.

into growth provides an explanation of those clinical instances in which cancer appears after certain injuries of tissues that had previously seemed normal

SUMMARY

In order to investigate some of the biologic properties of carcinogens and to gain further information on the various stages of cancer formation, methylcholanthrene dissolved either in benzyl alcohol or in wool fat was applied at various periods to the skin of 240 albino mice. Wool fat had a pronounced retarding influence on the formation of cutaneous tumors, and its effect was in direct contrast to the rapid onset of neoplasms observed when benzyl alcohol was used as the solvent for the hydrocarbon. When a solution of methylcholanthrene in wool fat was painted on mice for four months, no visible tumors were found, but when the carcinogen was subsequently applied to the same area in a solution of benzyl alcohol, tumors quickly appeared. The rapidity of appearance of perceptible neoplasms suggested that the carcinogen merely stimulated the proliferation of existing tumor cells during the second period of treatment, a concept which gained further support from the fact that croton oil, a noncarcinogenic substance, could be substituted for methylcholanthrene during the second stage with similar results. Wool fat greatly diminished the effects of both methylcholanthrene and croton oil, a fact implying that it had a neutralizing effect on these tissue irritants.

The results of this experiment suggest at least two biologic characteristics of methylcholanthrene that are essential for tumor development, one is responsible for the genesis of the neoplastic cell, and the other stimulates cellular multiplication. The latter property appears to be the result of nonspecific irritation of tissue. Neoplasms develop only as a consequence of the proper sequential summation of such properties.

Further support is given to the suggestion that tumor formation may be divided into various phases as follows:

1. **Period of induction.** During this period the neoplastic cell is formed. For certain tumors this phase is further subdivided into a stage of preneoplasia and a stage of genesis.
2. **Critical period or period of reversibility.** This is a transitional period in which the growth of the cells is in delicate equilibrium with the influence of their environment and depends on the balance between the proliferative capacity of the cell and the local tissue resistance.
3. **Period of progression.** During this period growth is relatively unchecked.

Notes and News

The Scientific Exhibit, Atlantic City Session of the American Medical Association—At the Atlantic City Session, June 9 to 13, 1947, the American Medical Association will observe its centennial anniversary. For almost half of those hundred years—since 1899—the Scientific Exhibit has been a feature of each annual session and has developed into a short course in graduate medical instruction.

Exhibits at the 1947 session will cover all phases of medicine. A certain amount of historical material will be included, but emphasis will be placed on the latest developments of medical science. The representative to the Scientific Exhibit from the Section on Pathology and Physiology is Dr. Frank W. Konzelmann, Atlantic City Hospital, Atlantic City, N. J.

Applications for space should be submitted as early as possible, since the closing date is Jan. 13, 1947. Application blanks may be obtained either from Dr. Konzelmann or from the Director, Scientific Exhibit, American Medical Association, 535 North Dearborn Street, Chicago 10.

Appointments, Etc—Frank B. Queen, formerly pathologist at Passavant Hospital, Chicago, and then director (colonel, United States Army) of the laboratories at Bushnell General Hospital, Brigham, Utah, is now professor of pathology in the University of Oregon Medical School. He has charge of the tumor clinic of the school and directs the activities of the Oregon division of the American Cancer Society.

R. D. Johnston, recently on duty in the Army Institute of Pathology, has been appointed assistant professor of pathology in the University of Oregon Medical School.

Brigadier General Standhope Bayne-Jones has resumed work at Yale University as professor of bacteriology and director of the Jane Coffin Childs Memorial Fund for Medical Research.

At Northwestern University Medical School J. P. Simonds has become professor of pathology emeritus. His successor is William B. Wartman, formerly assistant professor of pathology in Western Reserve University. Thomas C. Laipply, also assistant professor at Western Reserve University, has been appointed associate professor of pathology at Northwestern University.

F. B. Johnson has resigned as professor of clinical pathology at the Medical College of the State of South Carolina.

At the University of Washington School of Medicine, Seattle, a department of microbiology has been established, which includes bacteriology, immunology, virology, mycology and medical protozoology. The director is Charles A. Evans, associate professor of bacteriology and immunology, University of Minnesota.

Death—Ward J. MacNeal, professor of bacteriology at the New York Post-Graduate Medical School and Hospital, Columbia University, died Aug. 15, 1946, 65 years old.

Awards—Anton J. Carlson, professor emeritus of physiology at the University of Chicago, received the Distinguished Service Medal of the American Medical Association at its recent meeting in San Francisco.

The Ward Burdick Medal of the American Society of Clinical Pathologists has been awarded to A S Wiener and Philip Levine for their work on the Rh factor

The Trudeau Medal of the National Tuberculosis Association has been awarded to Max Pinner, Berkeley, Calif, for his contribution to the control of tuberculosis

The Walker prize of the Royal College of Surgeons is awarded every five years "to the person, if any, who shall be deemed to have done the best work during the preceding five years in advancing the knowledge of the pathology and therapeutics of cancer" The prize for the years 1936 to 1940, inclusive, was awarded to F P Rous, of the Rockefeller Institute for Medical Research, who received it on July 10 last, when he lectured on antecedents of cancer (*Lancet* 2 98, 1946)

American Board of Pathology—A H Sanford, Rochester, Minn, Frank Hartman, Detroit, Frederick Lamb, Davenport, Iowa, and J J Moore, Chicago, have retired after twelve years of service New members of the board are J A Kasper, Detroit, James Kernohan, Rochester, Minn, Edwin Schultz, San Francisco The other members of the board are Shields Warren, Boston, N C Foot, New York, R P Custer, Philadelphia, William Sunderman, Philadelphia, Paul Cannon, Chicago, James McNaught, San Francisco, R A Moore, St Louis N C Foot is the president, M Sunderman, vice president, and R A Moore, secretary-treasurer The next examination will probably be held in Philadelphia about June 1, 1947 Inquiries should be sent to Dr R A Moore, Washington University School of Medicine, St Louis 10

Finney-Howell Fellowships—Applications for 1947 must be filed by January 1 next (1211 Cathedral Street, Baltimore) The awards will be made March 1, 1947 The purpose of the fellowships, each \$2,000 a year, is to support research into the causes and the treatment of cancer in places approved by the directors

National Institute of Health—The first session of the pathology study section of the institute's research grants division was held Aug 16, 1946 This section, which is one of more than twenty groups of consultants advising in the whole field of medical research, is composed of Paul Cannon, chairman, W S DeMonbreun, W H Feldman, W D Forbus, H Goldblatt, J S McCartney, A R Moritz, A Rich, J F Rinehart, H P Smith, representatives of the Surgeons General of the United States Army, the United States Navy and the medical department of the Veterans Administration, and R D Lillie, National Institute of Health, Bethesda, Md, executive secretary

Application forms for grants in aid may be obtained by addressing the Chief, Research Grants Division, National Institute of Health, Bethesda 14, Md For prompt action applications should be filed well in advance of the quarterly meetings of the study section concerned

Books Received

LE REIN POLYKYSTIQUE ETUDE MORPHOLOGIQUE, CLINIQUE ET PHYSIOPATHOLOGIQUE By P P Lambert, Clinique medicale Hopital universitaire Saint-Pierre (Prof Paul Govaerts) et Laboratoire d'Histologie, Universite de Bruxelles (Prof P Gerard) Preface by Prof Paul Govaerts Pp 140 Paris Masson & Cie, 1943

After a review of previous studies, mainly concerned with cystic kidney of the newborn, the author reports the results of an extensive and comprehensive morphologic study of the polycystic kidney of the adult. Because of technical difficulties, this subject had been incompletely studied previously.

The cysts are classified into three types: (a) the glomerular cysts, which are "closed cysts," (b) the tubular cysts and (c) the cysts of the collecting tubules. By serial sections and graphic reconstruction, the author shows that in the adult most of the cysts of the last two types are open cysts, while in the newborn they are closed cysts.

If it is assumed that there is no pathogenic difference between the cystic kidney of the newborn and that of the adult, the theory of absence of union between the metanephric and the ureteral parts of the nephron is refuted. Contrary to the views of some investigators, retention is not considered as playing a role in the formation of the cysts. The cysts are probably the cause of the failure of the two parts of the nephron to become united but are not the result of that failure. In the newborn most of the cysts are "closed cysts" because the cystic degeneration occurs before the union of the two parts of the nephron. In the adult they occur in an otherwise well developed nephron. The author, however, does not explain the pathogenesis of the primary cystic degeneration of the nephron.

The second part of the book comprises the results of a chemical analysis of the contents of the cysts of the kidneys. As judged by means of an intraperitoneal injection of inulin, it seems that most cysts in the adult are connected with a normally functioning glomerulus. The author believes, contrary to the opinion of other investigators, that the epithelium lining the tubular cysts still conserves its physiologic functions. This book, though somewhat controversial, is an interesting and stimulating study of the polycystic kidney.

RENAL HYPERTENSION By Eduardo Braun-Menendez, Juan Carlos Fasciolo, Luis F Leloir, Juan M Munoz and Alberto C Taquini, Institute of Physiology, Faculty of Medical Sciences and Institute of Cardiology, V F Greg Foundation, Buenos Aires, Argentina. Translated by Lewis Dexter, M D, Harvard Medical School and Peter Bent Brigham Hospital, Boston. Pp 451, with 93 illustrations. Price \$6.75. Springfield, Ill. Charles C Thomas, Publisher, 1946.

This volume is an excellent and complete review of the significant literature in the field of experimental and clinical renal hypertension by a group of Argentinian investigators who have contributed most importantly to the subject under the early stimulus of the famed physiologist Dr Bernardo A Houssay. Since the authors' interpretations of the available experimental and clinical evidence largely agree with the majority views of authorities, the book constitutes a sound summary of present knowledge of renal hypertension. This first English edition has been most competently translated by Dr Lewis Dexter, whose qualifications for the task include a period of scientific collaboration with the authors, as well as independent contributions to the subject.

The chapter divisions present the matter in a logical sequence from the methods of producing permanent experimental hypertension (including the classic work of

Goldblatt) to the relationships between experimental and clinical renal hypertension. Included are chapters on the pathophysiology and the pathogenetic mechanisms of experimental renal hypertension, the chemical, physiologic and pharmacologic properties of the renin-hypertensinogen-hypertensin-hypertensinase system and its relation to experimental renal hypertension, the protective action of the so-called normal kidney, the influence of the endocrine glands, and the role of other vasoconstrictor substances, including pressor amines. The authors offer a challenging classification of human hypertension, followed by chapters dealing with hypertensions of renal or probably renal origin and more importantly with essential and malignant hypertensions (which are classified as of possible renal origin). One chapter each is devoted to methods of medical and surgical treatment, which are conservatively and fairly evaluated.

Each chapter includes a concise summary, which should prove convenient to the reader who is new to the field and to the clinician who may wish to read in brief the early chapters devoted to experimental hypertension and in detail the later chapters concerned with clinical hypertension. An appendix of chemical and biologic methods useful to investigators and an inclusive yet selective bibliography complete this dynamic, vibrant volume, which should be read by all interested in the problem of hypertension.

PENICILLIN ITS PRACTICAL APPLICATION Under the general editorship of Prof. Sir Alexander Fleming, M.B., B.S., F.R.C.P., F.R.C.S., F.R.S., professor of bacteriology in the University of London, St. Mary's Hospital, London. Pp. 380, with 59 illustrations. Price \$7. Philadelphia: The Blakiston Company, 1946.

Here are six articles of a general nature about penicillin and twenty-one about different aspects of penicillin treatment, including its use in human dental and oral infections as well as in animal infections, all by British workers. Of the contributors of general articles, Fleming himself writes about the history and the development of penicillin and about the bacteriologic control of penicillin therapy. These articles and the preface have of course a very special interest. The other general articles deal with the pharmaceutic and the pharmacologic aspects and the methods of administration of penicillin. The illustrations are telling. The book gives an excellent conspectus of penicillin therapy as it exists today.

ARCHIVES OF PATHOLOGY

VOLUME 42

NOVEMBER 1946

NUMBER 5

1947,
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SUDDEN AND UNEXPECTED DEATHS OF YOUNG SOLDIERS

Diseases Responsible for Such Deaths During World War II

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BOSTON

AND

CAPTAIN NORMAN ZAMCHECK *

MEDICAL CORPS, ARMY OF THE UNITED STATES

AMONG the more than 40,000 autopsy protocols that were received at the Army Institute of Pathology between January 1942 and January 1946, there were many which concerned young and apparently healthy soldiers whose collapse and death from disease were so sudden and unexpected that there was little or no opportunity to make an antemortem diagnosis.

For several reasons this group of deaths was thought to provide a unique opportunity to study the kinds and special characteristics of those diseases which, by reason of either rapid evolution or sudden transformation of a latent abnormality into one incompatible with continued life, were most frequently responsible for the unexpected deaths of apparently healthy young adults.

One of the principal reasons for regarding the source material as unique was the fact that there was relatively little opportunity for a sick or indisposed soldier to escape medical surveillance for long in the military installations in continental United States, from which the majority of these deaths were reported. Although dispensary and hospital facilities were readily accessible, relatively few of these men had sought medical attention before their fatal seizure. Most of them either thought themselves to be in good health or were remarkably reticent about their ill health. It could be assumed that the majority had recently passed one or more complete physical examinations incident to induction into the Army, in the process of which neither a real nor a potential threat to health was recognized. It is difficult to see how a comparable series of cases could be collected from civilian sources.

From the Army Institute of Pathology, Washington, D. C.

* From the Department of Legal Medicine, Harvard Medical School

Statistical conclusions achieved in this paper were made with the assistance of Mr. Murray Geisler, Chief, Statistical Analysis Branch, Army Institute of Pathology.

The academic interest inherent in a study of sudden death from such well controlled source material is at once apparent, and there is also the possibility of obtaining practical information of military significance from such an investigation. It might be found that certain causes of unexpected death are peculiar to camp or barrack life. It might be disclosed that certain apparently insignificant prodromal disturbances had actually given warning of the impending disaster. Evidence might be adduced that certain physical or constitutional types are more susceptible than others to the kinds of degenerative disease or structural defects responsible for unexpected death. An answer might be suggested to the important question of whether or not there is a cause and effect relationship between strenuous physical exertion and death from disease.

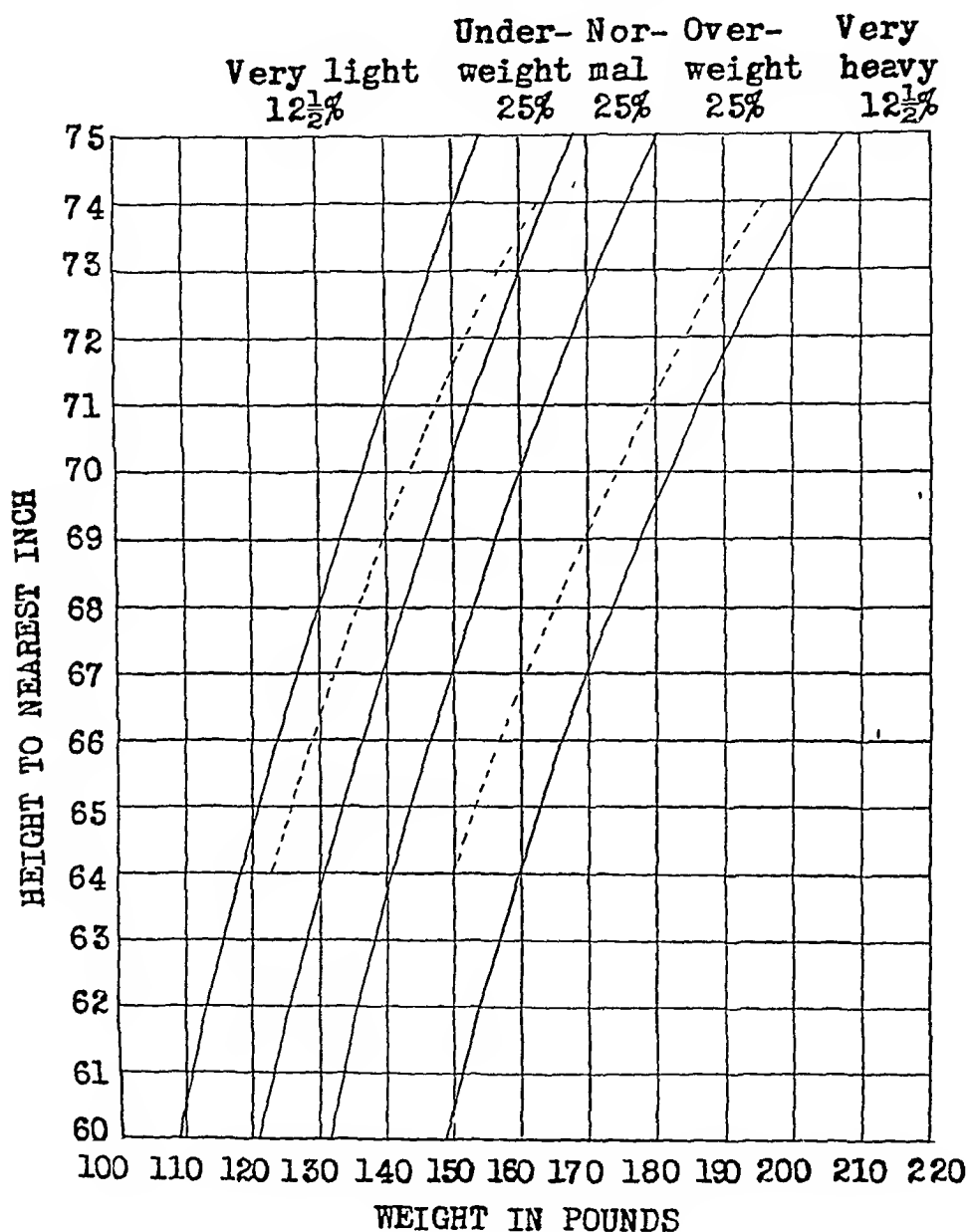
It soon became apparent that there were certain almost insurmountable difficulties to the making of a comprehensive statistical investigation of all causes of sudden death. One obstacle was the lack of uniform criteria for the determination of what constituted "sudden death." Therefore, to ascertain that this study included all deaths which had occurred within twenty-four hours after the onset of incapacitating symptoms would have required the reexamination of practically all protocols that had been received over this period. The alternative elected was to determine the various diseases that were responsible for unexpected deaths and then to investigate in some detail the diseases most commonly implicated.

Early in the investigation it became apparent that the body weight of many of those who died unexpectedly from disease was greater than that of the average soldier. In order to determine the extent to which the body weight in various categories of unexpected death deviated from the average, information pertaining to the stature and the body weight of all inductees was obtained through the statistical department of the Office of the Surgeon General.

The percentile distribution of all inductees in relation to body weight and stature is shown in the chart. The weight range of the 25 per cent of all soldiers that was central will hereafter be designated as "normal." The weight range that included the next 25 per cent of all soldiers that were heavier than "normal" will be designated as "overweight," and the next 25 per cent that were lighter than "normal" as "underweight." The extremes, which included the heaviest and lightest 12.5 per cent of all soldiers, will be referred to as "very heavy" and "very light." The Army's data on weight and stature of inductees correspond closely to statistics issued by the Metropolitan Life Insurance Company¹ for men over 25 years of age. The upper and lower

¹ Ideal Weights for Men, *Statist Bull Metrop Life Insur Co* 24:6 (June) 1943

limits of the ideal weight range for civilian men are indicated by the dotted lines in the chart. Whereas the Army has designated the central 25 per cent of inductees as normal, approximately 40 per cent of them fall within the "ideal" weight range for civilians.



The solid lines indicate the percentile distribution of all inductees during World War II according to weight and stature. The interrupted lines indicate the upper and lower limits for ideal weight according to stature of men over 25 as published by the Metropolitan Life Insurance Company.

The principal difficulty involved in interpreting the relationship of the data on weight to the incidence of disease is in the obtaining of accurate data at the autopsy table. Since these weights are largely estimates rather than true measurements, a sizable error may arise, obscuring such relationships as may exist.

In order to determine the extent to which autopsy room estimates of weight correspond with the measured weights of living soldiers, an analysis was made of data derived from the autopsy protocols of 296 soldiers who died of immediately fatal accidental injuries. In 180 of these, death resulted from gunfire, and in 116, from traffic accidents. These soldiers were characterized as very light, underweight, normal, overweight or very heavy after their body weight-body length relationships had been compared with those of the Army at large.

There is no reason to believe that the weights of this special group actually differed from those of the Army as a whole. The weight distribution of soldiers of this control group is compared with that of the entire Army in table 1. It is apparent that there is a great discrepancy between the estimated weights derived from autopsy protocols and the measured weights of inductees. It is not reasonable to believe that accidentally incurred fatal gunshot or traffic injuries occurred so seldom in underweight and with such disproportionate frequency in overweight soldiers as is indicated in table 1. A more plausible explanation of

TABLE 1—*Weight Distribution of Soldiers (Inductees and Control Groups)*

| Weight | Control Group of 296 Soldiers Dead of Accidents, per Cent | Inductees, per Cent |
|-------------|---|------------------------|
| Very light | 3.5 | 12.5 |
| Underweight | 10.5 | 25.0 |
| Normal | 23.0 | 25.0 |
| Overweight | 42.6 | 25.0 |
| Very heavy | 20.4 | 12.5 |

this discrepancy is either that pathologists habitually tend to overestimate the weights of dead bodies or that the average soldier is heavier than the average inductee.

The following criteria were observed in the selection of cases for this study: (a) the soldier who died unexpectedly was under 40 years of age, (b) he was ambulatory and presumably not suffering from a dangerous disease at the time of his fatal seizure, (c) the interval of time between the onset of incapacitating symptoms and death was less than twenty-four hours, (d) postmortem examination disclosed that death had resulted from recognizable disease or (e) that the pathologic changes were inadequate to account for death (excluding those cases in which trauma and poisoning, including alcohol, were considered probable because of anamnestic information and/or toxicologic analyses).

It was discovered that the principal categories of disease responsible for sudden death were heart disease, intracranial hemorrhage, meningococcemia, miscellaneous diseases and cause of death not disclosed by autopsy.

HEART DISEASE

Organic heart disease was estimated to have been responsible for between 200 and 300 unexpected deaths² It was estimated that more than 200 of these were due to coronary arteriosclerosis and at least 34 to other cardiac diseases

Coronary Arteriosclerosis

A group of 115 unexpected deaths from disease of the coronary arteries was selected for study, in the belief that it constituted a representative sample of deaths from this cause

TABLE 2—*Age Distribution in Cases of Sudden Death from Coronary Arteriosclerosis*

| Age, Years | Unexpected Deaths from Coronary Disease, per Cent | Total Army Personnel, per Cent |
|------------|---|--------------------------------|
| Under 20 | 0 | 9 |
| 20-24 | 8 | 38 |
| 25-29 | 14 | 30 |
| 30-34 | 44 | 16 |
| 35-39 | 34 | 6 |

Age—The distribution of sudden deaths from coronary disease according to age in relation to that of the Army as a whole is given in table 2 It is apparent that the incidence of fatal coronary disease rises rapidly with age

Race—Only 6 of the 115 unexpected deaths from this disease occurred in Negroes Although the total number is relatively small,

TABLE 3—*Weight Distribution in Cases of Sudden Death from Coronary Arteriosclerosis (92 Cases)*

| Weight | Sudden Coronary Deaths, per Cent | Accidental Deaths, per Cent |
|-------------|----------------------------------|-----------------------------|
| Very light | 2.9 | 3.5 |
| Underweight | 16.0 | 10.5 |
| Normal | 17.0 | 23.0 |
| Overweight | 41.0 | 42.6 |
| Very heavy | 23.0 | 20.4 |

since the ratio of Negroes to white persons in the Army was approximately 1/9, this may provide some indication of a lesser susceptibility of Negroes to sudden death from disease of the coronary arteries

Obesity—The relation of body weight to unexpected death from coronary disease is given in table 3 It appears that the incidence of obesity as based on autopsy room estimates of weight is no higher in

² A complete study of deaths from disease of the coronary arteries in soldiers aged 20 to 39 is being prepared by Dr Wallace Yater, civilian consultant to the Army Institute of Pathology, and associates

soldiers dead of coronary disease than in those dead of accidental mechanical violence

Exertion—In 98 instances the type of physical activity that had preceded or accompanied the onset of the fatal seizure was recorded. The relation of physical exertion to death from coronary disease is shown in table 4.

If the onset of acute heart failure due to coronary insufficiency were unrelated to any extrinsic factor, the incidence during any given interval of time throughout the day or the night should be proportional to the duration of that interval. If such were the case and if the soldiers spent an average of one third of their time sleeping, it might be expected that the onset of acute heart failure would have taken place during sleep in approximately one third of the deaths. It may be seen that the onset of acute heart failure occurred during sleep in only 15 per cent of the cases. In 29 per cent the onset of heart failure coincided with or immediately followed strenuous physical exertion. If the estimate

TABLE 4—*Relationship of Physical Activity to Onset of Fatal Seizure (98 Cases)*

| Type of Activity | Estimated Time Devoted by Soldier to Inactivity and to Activity, per Cent | Fatal Seizures Coincident with Inactivity or Activity, per Cent |
|--------------------------|--|--|
| Sleep | 33 | 15 |
| Inactivity—subject awake | 17 | 21 |
| Ordinary activity | 33 | 35 |
| Strenuous exertion | 17 | 29 |

is correct that not more than 17 per cent of the average soldier's twenty-four hour day was devoted to strenuous physical activity, it appears that the number of those whose fatal seizures occurred incident to exertion was in excess of that which would be expected by random distribution. It is not likely that all cases in which the onset of acute heart failure was related to physical exertion are actually included in this 29 per cent. It could not be determined how many of those who collapsed during rest or during ordinary activity had become recently inactive or less active because of increasing physical disability. Thus there were a number of instances in which a soldier, found dead in bed, was reported as having been "below par" or "not feeling well" on the previous day. Usually such a record did not indicate whether the initial indisposition occurred during rest or during a period of unusual physical exertion. Thus, although the onset of the attack in 29 per cent of the cases of unexpected death from coronary disease was known to be related to exercise, the actual coincidence may have been considerably greater.

It is recognized that data of this kind do not prove or disprove a cause and effect relationship between physical activity and acute heart

failure. However, they do suggest that if a young man is to die unexpectedly of coronary disease, the onset of the terminal attack of acute heart failure is more likely to take place during strenuous physical exertion than during sleep.

Survival Time After Onset of Fatal Seizure—In 75 instances it was explicitly stated that death occurred within a few minutes or less after the onset of incapacitating symptoms. These represented soldiers who were regarded as healthy or at least as not dangerously ill until a few minutes before death. In 20 instances the onset of incapacitating symptoms was survived for periods that ranged between fifteen minutes and twenty-four hours. There were 20 soldiers who were found dead in circumstances that made it impossible to estimate the exact interval between fatal seizure and death, although it was known to be less than twenty-four hours.

Nature of Fatal Seizure—The precise nature of the fatal seizure was either unknown or not recorded in 27 instances. In 42 it consisted in an unexpected syncopal attack, if there were prodromal symptoms, they were either not recorded or not complained of. In 2, convulsions followed loss of consciousness. In 44 instances the soldier complained of pain (precordial or epigastric) or both pain and vomiting immediately before loss of consciousness.

Past History—As previously indicated, these 115 instances represented cases of unexpected death of a soldier who, so far as could be learned, was not recognized to be suffering from coronary disease. In 94 cases, the patient thought that he had been well prior to the time of his fatal seizure, he had not complained or information regarding his previous symptoms was not recorded in the history of the case. In 21 there was a record of previous symptoms which in retrospect could be attributed to coronary disease. Nineteen soldiers had suffered previous attacks of precordial or epigastric pain, the latter sometimes being regarded as indigestion. Three of these soldiers were also known to have complained of shortness of breath or excessive fatigability. Two, who had apparently not complained of previous attacks of pain, had a history of fainting spells on one or more occasions.

Pathologic Observations—In each of the 115 cases gross and microscopic examinations of the heart and other organs were made. In all instances the descriptions indicated that one or both coronary arteries were the seat of severe atherosclerosis. The sclerotic changes involved the main arterial trunks in all but 2 cases, and in these the disease was described as affecting principally the small, intramuscular branches of the arteries. So far as could be determined from the protocols, the vascular lesions did not differ significantly from those observed in the coronary arteries of older persons, except that there was less calcifica-

tion Although subintimal hemorrhage was observed in several instances, occlusion of the lumen by this cause was not described In some the vessels were described as obliterated by sclerotic change, and in others the diseased vessels were occluded by thrombi The principal pathologic characteristics of this group are summarized in table 5

It may be seen in table 5 that in approximately half of the cases complete occlusion of a coronary artery was not found and that in less than a third was thrombosis demonstrated The finding that the ratio of left to right coronary occlusion is approximately 2.5:1 is in general accord with the observations of others

It is of interest that myocardial infarction was found in less than a fifth of these cases

TABLE 5—*Pathologic Changes Observed in 115 Cases of Sudden Death from Coronary Arteriosclerosis*

| Pathologic Change Observed at Autopsy | | |
|---|----|-----|
| Coronary thrombosis | | 31 |
| Left | 24 | |
| Right | 7 | |
| Obliterative coronary arteriosclerosis (without recognized thrombosis) | | 23 |
| Left | 14 | |
| Right | 9 | |
| Severe coronary arteriosclerosis (neither thrombosis nor obliteration observed) | | 61 |
| Total | | 115 |
| Myocardial infarction | | 22 |
| Recent | 15 | |
| Healed | 7 | |
| Myocardial fibrosis without recognized infarction | | 27 |
| No mention of myocardial pathologic change | | 66 |
| Total | | 115 |
| Cardiac hypertrophy | | |
| Heart weight known or inferred to be less than 400 Gm | 82 | |
| Heart weight over 400 Gm | 33 | |
| Total | | 115 |

In 82 instances the heart weight either was recorded to be less than 400 Gm or could be inferred to be from the description In only 2 instances was the heart weight reported in excess of 450 Gm

Characteristically the lungs and the abdominal organs were described as being the seat of acute passive hyperemia Although heavy lungs were almost invariably observed when the interval between collapse and death had been longer than a few minutes, there were a number of instances in which severe pulmonary edema was observed in a soldier whose collapse and death were described as almost instantaneous

Although no attempt was made to review all the histologic material, random sampling confirmed the observations of Allen³ in regard to the high frequency of splenic eosinophilia in cases of sudden death

In 4 instances of sudden death from coronary disease there was both gross and microscopic evidence of agonal or postmortem bleeding

3 Allen, A. C. Arch Path 37 20, 1944

throughout the head and body of the pancreas. In 1 of these, death had occurred within a few minutes after the onset of the fatal seizure. There was no apparent relationship between the presence of pancreatic hemorrhage and the length of the interval between death and postmortem examination.

Other Forms of Heart Disease

There were 34 cases in which death was due to some form of heart disease other than coronary arteriosclerosis. These fell into the following categories:

Acute and Subacute Isolated Myocarditis—In 14 instances death of acute heart failure resulted from diffuse or focal exudative inflammation of the myocardium. The ages of the subjects ranged between 18 and 36 years, 12 were white, and 2 were Negroes. Ten of the 14 died within a few minutes after an unexpected syncopal attack.

In 2 soldiers the fatal seizure occurred during a period of strenuous physical exertion, and 8 were at rest or engaged in ordinary activity. Four were found dead in bed. Five of the 14 were reported to have been below par for a day or so prior to the occurrence of the fatal seizure, although none had been confined to bed. The records did not indicate that any of the 14 recently had been hospitalized or had suffered from an infectious disease more serious than a cold. Postmortem examinations were reported to have disclosed no significant pathologic changes other than myocarditis.

Syphilitic Aortitis—In 6 cases of sudden death active syphilitic inflammation of the aortic ring was found at postmortem examination. In 3 there was complete occlusion of a coronary ostium, in 1 a nondeforming aortic valvulitis and in another aortitis with dilatation of the aortic ring. All 6 of the soldiers were over 30 years of age, 4 were Negroes and 2 were white persons. None of them was known to have complained of not feeling well prior to the time of the fatal seizure. Two were found dead in bed, and the other 4 died within a few minutes after unexpected syncopal attacks which occurred during periods of ordinary activity. None of the protocols contained a description of any lesion that could be construed as syphilitic myocarditis.

Rheumatic Heart Disease—The unexpected deaths of 5 soldiers were attributed to rheumatic heart disease. Acute cardiac dilatation was found in all 5, but only one heart weighed more than 400 Gm. Chronic valvulitis was present in all 5, but in only 1 was significant valvular deformity (mitral stenosis) described. In 1 of the 5 the microscopic observations indicated active rheumatic carditis. One was found dead in bed, and the other 4 collapsed unexpectedly during periods of physical exertion.

Idiopathic Myocardial Fibrosis—Five deaths were attributed to acute cardiac failure due to extensive myocardial fibrosis which was not associated with coronary disease, exudative myocarditis or recognizable rheumatic stigmas. In 3 of the 5 cases there was cardiac hypertrophy. In none were abnormalities of the epicardium, the endocardium or the valves noted.

INTRACRANIAL HEMORRHAGE

In at least 91 cases unexpected death resulted from nontraumatic intracranial hemorrhage. It was estimated that these 91 comprised approximately a third of all cases of spontaneous intracranial hemorrhage that were reported to the institute between 1941 and 1945.

Subarachnoid Hemorrhage from Congenital, Berry or Miliary Aneurysms

In 48 instances of unexpected death it was proved and in 21 instances it was inferred that death had resulted from the rupture of a thin-walled saccular aneurysm of the type described by Forbus,⁴ Bremer⁵ and others as congenital, berry or miliary. In the 21 instances in which the subarachnoid hemorrhage was presumed but not proved to have originated from a berry aneurysm, a careful examination of the brain and its vessels had failed to disclose the nature of the vascular defect. It must have been small, and in view of the fact that there was (a) no history or evidence of antecedent trauma, (b) no systemic disease and (c) no other type of abnormality of the brain or the meninges, rupture of a miliary aneurysm was regarded as the probable cause of death. In several of these, one or more intact congenital aneurysms of the circle of Willis were found, but the precise site of rupture was not identified.

A representative case of rapidly fatal subarachnoid hemorrhage which was presumed to have resulted from the rupture of a berry aneurysm is as follows:

A 29 year old soldier in apparent good health collapsed while running base during a ball game. He got up with difficulty and staggered to the base, where he collapsed, gasped a few times and died. Close questioning of associates failed to disclose any previous complaints or abnormal actions. He was never known to have reported for sick call. There was no history of syphilis, other disease or injury.

When the cranium was opened, an extensive subarachnoid hemorrhage was observed, and there was a small amount of firm clot in the right side of the sylvian fissure, in the vicinity of the middle cerebral artery. No vascular defect was found, and there was no other evidence of cerebral vascular disease.

Age—The ages of the soldiers in this series whose deaths were known or inferred to have resulted from rupture of a superficial congenital aneurysm are given in table 6. If the 65 cases comprising this

4 Forbus, W. D. Bull. Johns Hopkins Hosp. **47**: 239, 1930.

5 Bremer, J. L. Arch. Path. **35**: 819, 1943.

group constitute a representative sample of cases of sudden death from rupture of a superficial congenital aneurysm it appears that the frequency of sudden death in such cases tends to increase with age between 19 and 39

Race—Of 69 soldiers who died of subarachnoid hemorrhage due to rupture of a berry aneurysm, 66 were white and 3 were Negroes

Obesity—The relation of body weight to death from rupture of a berry aneurysm was as given in table 7 Although a slightly higher

TABLE 6—*Age Distribution in Cases of Sudden Death from Rupture of Superficial Congenital Aneurysms (65 Cases)*

| Age, Years | Unexpected Deaths from Ruptured Aneurysms, per Cent | Total Army Personnel, per Cent |
|------------|---|--------------------------------------|
| Under 19 | 5 | 9 |
| 20 24 | 14 | 33 |
| 25 29 | 44 | 30 |
| 30 34 | 21 | 16 |
| 35 39 | 15 | 6 |

TABLE 7—*Weight Distribution in Cases of Sudden Death from Rupture of Berry Aneurysms (53 Cases)*

| Weight | Sudden Deaths Due to Rupture of Berry Aneurysms, per Cent | Accidental Deaths, per Cent |
|-------------|---|--------------------------------|
| Very light | 1 | 3.5 |
| Underweight | 2 | 10.5 |
| Normal | 28 | 23.0 |
| Overweight | 49 | 42.6 |
| Very heavy | 20 | 20.4 |

TABLE 8—*Relation of Physical Activity to Rupture of Congenital, Berry or Military Aneurysms (54 Cases)*

| Type of Activity | Estimated Time Spent in Inactivity or Activity, per Cent | Fatal Seizures Coincident with Inactivity or Activity, per Cent |
|--------------------------|--|--|
| Sleep | 33 | 17 |
| Inactivity—subject awake | 17 | 29 |
| Ordinary activity | 33 | 29 |
| Strenuous exertion | 17 | 24 |

incidence of sudden death due to ruptured aneurysm is suggested in the "normal" and "overweight" categories than in the corresponding accidental death groups, the number of cases is too few for this to be of statistical significance

Physical Exertion—Although the series was scarcely large enough to justify conclusions regarding the relationship of physical exercise to the onset of the fatal seizure, the findings may be summarized as in table 8 It is apparent that there was no statistical evidence to

indicate that physical exertion predisposed to rupture of congenital aneurysms. However, when account is taken of the extreme fragility of these lesions, it seems obvious that a sudden sharp rise in blood pressure might well precipitate rupture. Although there was no instance in which an aneurysm was thought to have been caused or ruptured by direct cranial trauma, the onset of fatal hemorrhage in 9 instances was observed to have been coincident with such exercise as base running in a ball game, wrestling, tennis, drilling, pushing a piano, changing a flat tire, golf, badminton and swimming. The inference that such activities are probably dangerous for a person having a congenital defect in a cerebral vessel is inescapable.

Survival Time After Onset of Fatal Hemorrhage—In 44 cases of superficial cerebral aneurysm in which the onset of the fatal seizure was witnessed, the average interval between loss of consciousness and death was six hours. It should be borne in mind that the series was arbitrarily limited to cases in which the death occurred within twenty-four hours after the time that the fatal hemorrhage either was recognized or caused loss of consciousness. When the hemorrhage was of the burrowing type, the average survival time was between nine and ten hours, whereas the average survival time when hemorrhage was exclusively extracerebral was less than four hours. Approximately one third of the persons whose hemorrhage was not of the burrowing type died within an hour after loss of consciousness, whereas only 1 whose hemorrhage was in part parenchymatous and in part subarachnoid died during the first hour.

Another matter of interest in relation to time of survival after the onset of the terminal hemorrhage was the presence or the absence of blood in the lateral ventricles. There were at least 30 instances of primary subarachnoid hemorrhage in which the only way that blood could have regained access to the lateral ventricles was by following the normal anatomic route through the fourth ventricle, the aqueduct and the third ventricle. In 21 of these the postmortem examination disclosed blood throughout the ventricular system, whereas in 9 it was specifically stated that the ventricles did not contain blood. When death occurred rapidly (within two hours) after the onset of the fatal hemorrhage, blood was found to have reached the lateral ventricles of some patients and not those of others. Blood was found in the lateral ventricles of all who had survived more than two hours after the onset of the subarachnoid hemorrhage.

Character of Fatal Seizure—In the majority of cases in which the fatal seizure was witnessed, loss of consciousness was immediately preceded by headache and vomiting and was followed by fecal and urinary incontinence.

Among 69 cases of unexpected death from rupture of a congenital aneurysm there were only 3 in which clinical proof of leakage of blood had appeared before the fatal rupture. In these 3, lumbar puncture disclosed blood in the spinal fluid twelve days, two months and six months respectively before the occurrence of the fatal hemorrhage. It might be argued that the deaths in these cases should not be regarded as unexpected. At the time of the fatal seizure, however, the soldiers not only were ambulatory but were thought to be in good health.

In addition to these 3 there were 16 other cases in which there was anamnestic evidence of cerebral disturbance in advance of the fatal rupture. The most common symptom was headache, which varied in severity, duration and periodicity in the various cases. In some the first headache of which there was record occurred only a few days before death, whereas in others headache had been complained of for many weeks or months. Other anamnestic evidence of intracranial disease included attacks of nausea and vomiting, syncope, convulsions, stiff neck, malaise, emotional disturbances and evanescent nerve palsies. The last-mentioned symptoms were reported in 4 instances, and in all 4 the autopsy disclosed an adherent aneurysm with burrowing hemorrhage.

In 54 cases, either there were no previous signs or symptoms of intracranial disease or the circumstances were such that no anamnestic information was available.

Attention has already been called to the fact that hemorrhage from some of these superficial aneurysms infiltrated and destroyed the adjacent brain tissue whereas the bleeding from others was entirely external. It may or may not be significant that previous or prolonged symptoms of intracranial hemorrhage were present in almost a third of the patients who had burrowing hemorrhages and in only a negligible number of those in whom the hemorrhage was exclusively extracerebral.

It appears that when blood is extravasated into the adjacent brain substance through rupture of a superficial aneurysm the first hemorrhage is less likely to be fatal than when the blood escapes directly into the subarachnoid space. This observation lends support to the hypothesis that a burrowing hemorrhage is the result of some organic fixation of the aneurysm to the adjacent cerebral surface.

Pathologic Observations—The arterial distribution of the 48 recognized ruptured aneurysms is shown in table 9.

In 6 instances the lesions were multiple, there being one ruptured and one or more unruptured aneurysms of the circle of Willis in the same person. The anatomic distribution of the aneurysms observed in this series was similar to that reported by other authors (McDonald and Korb,⁶ Martland⁷ and Forbus⁴). Although a frequent site of

6 McDonald, C A, and Korb, M. *Arch Neurol & Psychiat* 42: 298, 1939.

7 Martland, H S. *Am J Surg* 43: 10, 1939.

aneurysm formation was at a vascular junction or bifurcation, such a relationship was not always recorded. In several instances the information available was so scanty that the precise location of the aneurysm on a given vessel could not be determined. Considerably more than half of the aneurysms were found on or anterior to the middle cerebral arteries.

The size of the ruptured aneurysms ranged from 0.3 to 3.0 cm in diameter, and the defects responsible for fatal hemorrhage varied from what was described as a pinpoint hole to a ragged blow-out of the entire dome of the sac. All aneurysms were described as thin-walled, and in cases in which microscopic examinations were recorded mention was made of the absence of elastic fibers and media. Some were partially occluded by thrombi, but the majority were empty and smooth lined.

TABLE 9—*Anatomic Distribution of Forty-Eight Ruptured Aneurysms*

| | Aneurysms |
|---|-----------|
| Anterior cerebral artery and its branches | 5 |
| Junction of anterior cerebral and anterior communicating arteries | 8 |
| Junction of anterior cerebral and middle cerebral arteries | 3 |
| Middle cerebral artery and its branches | 15 |
| Junction of internal carotid artery and circle of Willis | 2 |
| Junction of middle cerebral and posterior communicating arteries | 0 |
| Posterior communicating artery | 1 |
| Junction of posterior communicating and posterior cerebral arteries | 2 |
| Posterior cerebral artery | 3 |
| Junction of posterior cerebral and basilar arteries | 3 |
| Cerebellar artery | 1 |
| Basilar artery | 2 |
| Junction of basilar and vertebral arteries | 1 |
| Vertebral artery | 2 |
| Total | 49 |

Although several ruptured aneurysms were described as partially surrounded by organized blood clot and some were associated with hematogenous pigmentation of the surrounding meninges, there was poor correlation between clinical and pathologic evidence of bleeding prior to the time of the fatal rupture. This lack of correlation is presumed to be caused by incomplete clinical information in the protocols rather than to freedom from symptoms on the part of those suffering the bleeding.

It was apparent that two types of intracranial hemorrhage resulted from the rupture of superficial aneurysms. In one the hemorrhage was entirely external, with no blood being extravasated into the substance of the brain. In the other there was bleeding into the adjacent brain tissue as well as into the subarachnoid space. There were 46 instances of the former and 22 of the latter.

In the cases in which there was parenchymatous as well as subarachnoid hemorrhage, there was great variation in the extent to which brain tissue had been destroyed. In some the hemorrhage was con-

fined to a relatively narrow zone of the adjacent cortex, whereas in others there was deep extravasation, sometimes communicating with the nearest ventricle. In all but 1 case the hemorrhage involved a cerebral hemisphere. In that instance there was superficial destruction of one lobe of the cerebellum following rupture of an aneurysm of a vertebral artery.

A typical instance of burrowing aneurysm is as follows:

A 34 year old white medical officer experienced sudden severe pain over the right eye, followed by headache, nausea, vomiting and sweating, while walking to the post exchange. He was found to have a facial weakness on the left side, with widely dilated pupils. The fundi were normal. There was diminished auditory air conduction on the left. There was no stiffness of the neck, and the Babinski reflex was normal. The blood pressure was 128 systolic and 60 diastolic. One hour later he appeared confused but claimed that he was free of pain and felt fine. Six and a half hours after the onset of symptoms he was awakened by the return of severe pain over the right eye, he immediately became unconscious, had convulsive movements, was incontinent and three hours later became cyanotic and died.

Inquiry into the previous history revealed that five and one-half months earlier he suddenly had an agonizing pain in his neck, followed by headache and projectile vomiting. He was hospitalized, and a lumbar puncture at that time disclosed numerous red blood cells in the spinal fluid. During his hospital stay of fifteen days a slight paralysis became evident on the lower part of the left side of the face. The headache disappeared, and the patient was discharged to duty, with a diagnosis of subarachnoid hemorrhage of undetermined cause. Six weeks before death he had an evanescent attack of numbness of the left side of the tongue and drooping of the left facial muscles. For twenty-four hours following this attack he had a mild headache.

The autopsy disclosed subarachnoid hemorrhage originating from a small sacular aneurysm, 8 mm in diameter, located at the junction of the right middle cerebral artery and a branch that extended toward the island of Reil. The aneurysm had ruptured into the inferior surface of the tip of the temporal lobe, and the parenchymatous hematoma communicated with the inferior horn of the lateral ventricle.

Although studies of the pathologic anatomy did not yield the reason why rupture of some aneurysms resulted in parenchymatous hemorrhage whereas rupture of others did not, they did suggest one explanation sufficiently plausible to warrant consideration. This is that the pressure of an enlarging aneurysm on the surface of the brain or the slow escape and organization of blood in the space between the aneurysm and the brain is undoubtedly capable of eliciting sufficient reactive inflammation to establish adhesions. If the rupture of such an adherent aneurysm occurs through the portion of the wall that is adherent to the brain, it is reasonable to suppose that the blood escapes first into the brain substance and later breaks through the adhesions into the subarachnoid space. The frequent finding of a ragged blood-filled cavity between the super-

ficial aneurysm and the nearest ventricle suggests that this explanation may be valid

Subarachnoid Hemorrhage from Other Vascular Lesions

In 4 cases of primary subarachnoid hemorrhage bleeding resulted from some type of vascular lesion other than congenital, berry or miliary aneurysm

Cirsoid Aneurysm—A 32 year old white soldier was observed to be "thrashing around" on his bed at 8 p m and died forty minutes later. Friends stated that he had been "feeling badly" all day and had complained of headache and malaise.

At autopsy there was evidence of extensive subarachnoid hemorrhage, with bleeding into the left lobe of the cerebellum. The fourth ventricle was full of blood. The source of the hemorrhage was a lesion of the left anterior-inferior cerebellar artery, at first thought to be a cavernous hemangioma and later recognized as a cirsoid aneurysm. There was no other evidence of cerebral vascular disease.

Cavernous Hemangioma—A 32 year old obese white soldier had an unexpected convulsive seizure at 10 25 a m and died one and one-half hours later. He had complained of feeling weak at 9 a m but, so far as was known, had no previous complaints and had not reported for sick call.

Autopsy disclosed that both orbital plates were eroded by ovoid cavernous hemangiomas which were situated in part in the frontal fossa and in part in the posterior portions of the orbits. The tumors were deep purple and fluctuant, and one or both of them had ruptured, with resulting subarachnoid hemorrhage.

Angioma—A 22 year old white soldier, while engaged in ordinary activity, fainted and lapsed into deep coma. Pulmonary edema developed, and death followed eleven hours later. An antemortem lumbar puncture revealed blood in the spinal fluid. Associates reported that prior to the time that the soldier lost consciousness he had complained of headache, had vomited and had seemed restless and talkative.

At postmortem examination subarachnoid hemorrhage was found, which was due to the rupture of a "racemose venous-arterial angioma located in the hippocampal fissure."

Fusiform Aneurysm—A 38 year old soldier, who had been drinking during the evening, complained of feeling "ill," suddenly collapsed and died, without receiving medical attention.

Autopsy disclosed subarachnoid and intraventricular hemorrhage originating from a slitlike defect in the wall of the right internal carotid artery at its junction with the circle of Willis. The intracranial portions of both internal carotid arteries were the seat of fusiform aneurysms, and at the site of rupture medial hypoplasia was found on microscopic examination.

Intracerebral Hemorrhage from Aneurysms and Tumors

In 18 of the 91 cases of unexpected death due to intracranial hemorrhage the origin of the bleeding was an intracerebral vessel.

In 5 cases the vascular lesion was recognized macroscopically and confirmed microscopically as an aneurysm. In 3 cases the aneurysm was described as saccular. One aneurysm was mycotic, and another was fusiform and had apparently developed at the site of a congenital

medial defect In all 5 cases the aneurysm occurred on one or another branch of the middle cerebral artery, and in every instance the hemorrhage had ruptured into a lateral ventricle

Saccular Aneurysm—A 27 year old white soldier became nauseated and went to a latrine, where he vomited and collapsed. He died two and one-half hours later, without regaining consciousness Six weeks prior to death he was said to have had an attack of "grip" which was accompanied with severe frontal headache. Three weeks before death there had been a recurrence of headache

At the postmortem examination massive central hemorrhage was found in the left hemisphere, from a 4 mm saccular aneurysm situated on a ganglionic branch of the left lenticulostriate artery Distal to the aneurysm the lumen of the artery was partially occluded by a laminated thrombus The hemorrhage communicated with the lateral ventricle, and blood was found throughout the subarachnoid space There was no other evidence of cerebral vascular disease The heart weighed 430 Gm, but in view of the fact that the body weighed 89 Kg and measured 190 cm in length the heart weight was not considered abnormal Otherwise the postmortem findings were essentially normal

A 29 year old white soldier was found unconscious, with evidence of recent vomiting There were involuntary movements of the arms and the legs Blood pressure was first 40 systolic and 0 diastolic and was later observed to be 200 systolic and 70 diastolic The patient did not regain consciousness, acute congestive heart failure developed, and death followed two and one-half hours later

Postmortem examination revealed massive hemorrhage in the central portion of the right hemisphere, with rupture into the lateral ventricle On a striate branch of the right middle cerebral artery there was a small saccular aneurysm, which had ruptured Otherwise the postmortem findings were essentially normal The heart weighed 314 Gm

A 19 year old white soldier was found unconscious and died two hours later There was massive central bleeding into the left hemisphere, and a ruptured small saccular aneurysm was found on the lenticulostriate branch of the left middle cerebral artery Blood had escaped into the left lateral ventricle The heart weighed 280 Gm The postmortem examination failed to disclose any other significant abnormality

Fusiform Aneurysm—A 27 year old white soldier had a sudden syncopal attack accompanied by convulsions and died four and one-half hours later, without regaining consciousness

Postmortem examination disclosed massive intraventricular hemorrhage originating from a fusiform aneurysm of a branch of the left middle cerebral artery The wall of the aneurysm was described as "firm, yellow, and appears atherosclerotic." Although no weight was recorded, the heart was described as hypertrophic, with "minimal scarring of the musculature and some atheromatous plaques in the coronary arteries and aorta" No other significant abnormalities were noted

Mycotic Aneurysm—A 29 year old white soldier was sick in quarters with a "cold" and a furuncle of the anus He was thought to be doing well until early one morning when he was found in bed in a state of coma He died two hours later

About a year previously he had been hospitalized with a diagnosis of "meningitis, mild, type and cause undetermined" At that time he had suffered from severe frontal headaches and stiff neck. A lumbar puncture disclosed bloody spinal fluid

At postmortem examination there was extensive hemorrhage in the left hemisphere, communicating with the ventricular system. Several thrombotic vessels, the thrombi of which were estimated to be three to four weeks old, were found within the right internal capsule. In the right hemisphere, in the region of the claustrum, a thrombotic aneurysm of the right middle cerebral artery was surrounded by an extensive area of softening.

The terminal cerebral hemorrhage was assumed to have resulted from rupture of a mycotic aneurysm, embolic from the mitral valve. Also secondary to the bacterial endocarditis were an acute splenic tumor and a healed infarct of the kidney.

Intracerebral Angioma—There were 2 instances of unexpected death due to bleeding from an intracerebral angioma.

A 31 year old white soldier had an attack of headache and vomiting ten hours before death. There had been no recent history of disease or disability. A few minutes before death he had a convulsive seizure, with loss of consciousness.

Postmortem examination disclosed massive intraventricular hemorrhage due to a venous angioma in the floor of the anterior horn of the right lateral ventricle. Cholelithiasis was the only other significant abnormality.

A 19 year old Negro soldier collapsed while writing a letter and died two hours later, without regaining consciousness.

The only significant abnormality seen at postmortem examination was massive hemorrhage due to rupture of a venous angioma, with blood escaping into the right lateral ventricle.

Other Tumors—Two unexpected and rapidly fatal hemorrhages occurred in cystic brain tumors, one proved and the other presumed to be astrocytoma. One was cerebellar, and the other was in the right frontal lobe of the cerebrum. In one patient the onset of the fatal hemorrhage occurred during sleep and in the other during a period of strenuous exertion. One survived a few minutes and the other two hours after loss of consciousness. Neither had premonitory symptoms for more than a few minutes before loss of consciousness.

Intracerebral Bleeding—Cause Not Recognized

There were 13 instances of unexpected death due to central cerebral apoplexy in which neither the exact site nor the nature of the vascular defect was recognized. In all of them the hemorrhage was initiated and located in the lenticulostriate region. In 2 cardiac hypertrophy and diffuse vascular disease indicated chronic arterial hypertension. In 2 others there was chronic renal disease without cardiac hypertrophy or diffuse vascular disease. In 8 the hemorrhage had broken through into the lateral ventricle. The ages in this group varied between 20 and 30 years. The only unusual features presented by the patients was their age and their apparent freedom from prodromal symptoms of impending trouble. Most of them died within a few minutes after the onset of incapacitating symptoms. In some the fatal signs occurred during rest or ordinary activity, whereas others collapsed during or

after a period of strenuous physical exertion. The case described in the following abstract is representative of the group.

A 25 year old white corporal collapsed in the dental clinic and died four hours later. There was no other medical history.

Autopsy disclosed a large hemorrhage in the left lenticulostriate region, which had extended into the lateral ventricle. There were secondary hemorrhages in the pons and the medulla. Neither on gross nor on microscopic examination was there any other evidence of cerebral vascular disease. A complete postmortem examination failed to disclose any other significant gross or microscopic abnormality.

MENINGOCOCCEMIA

In 110 of the more than 350 cases of fatal meningococcic infections reported to the Army Institute of Pathology, death occurred within twenty-four hours after the onset of incapacitating symptoms. In 95 of these 110 cases the records were sufficiently complete for analysis.

In 80 of the 95 cases meningococci were recognized by culture or smear of either blood or cerebrospinal fluid. In the remaining 15 the

TABLE 10—*Age Distribution in Cases of Death from Meningococcic Infections*

| Age | All Meningococcic Deaths, per Cent | Sudden Meningococcic Deaths, per Cent | Total Army Personnel, per Cent |
|-------|---|--|--------------------------------------|
| 18-19 | 22.7 | 28.4 | 9.3 |
| 19-24 | 43.3 | 47.5 | 38.1 |
| 25-29 | 19.3 | 12.5 | 30.1 |
| 30-34 | 7.8 | 7.4 | 16.4 |
| 35-39 | 6.9 | 4.2 | 6.1 |

clinical and pathologic findings were regarded as sufficiently characteristic to justify the diagnosis of meningococcemia despite the absence of bacteriologic proof.

Age—The age distribution of the sudden deaths from this cause in relation to that of all meningococcic infections and to that of the Army as a whole is given in table 10.

It may be seen that whereas 47 per cent of the Army was comprised of men under 25 years of age 66 per cent of all men who died of meningococcic infections and 76 per cent of those who died suddenly from this cause were under 25. The incidence of rapidly fatal meningococcemia appears to be considerably higher in younger than in older soldiers.

Race—Fatal meningococcic infection was distributed among white and Negro soldiers as in table 11. It appears from the figures given in table 11 that the incidence of rapidly fatal death from meningococcic infections is greater in Negro than in white soldiers.

Weight and Height—In table 12 the body weight (corrected for height) of 69 men dying suddenly from meningococcic infection is

compared with the weight of a control group of 296 soldiers who died of accidental mechanical violence. It is apparent that there is no significant difference in weight between these groups.

TABLE 11—*Racial Distribution in Cases of Death from Meningococcic Infections*

| Race | Meningococcal Deaths | | | | Total Army Personnel, Per Cent |
|---------|----------------------|----------|--------|----------|--------------------------------|
| | Total | | Sudden | | |
| | Number | Per Cent | Number | Per Cent | |
| White | 296 | 84 | 74 | 78 | 90 |
| Negro | 49 | 14 | 19 | 20 | 10 |
| Others | 2 | 1— | 2 | 2 | 1— |
| Unknown | 5 | 1+ | | | |
| Totals | 352 | 100 | 95 | 100 | 100 |

Prodromal or Prefulminant Phase of the Disease—In 68 of the 95 cases it was recorded that the patient had been "below par" for varying periods prior to the onset of manifestations of incapacitating illness. The prodromal phase was usually characterized as a mild

TABLE 12—*Weight Distribution in Cases of Sudden Death from Meningococcic Infections*

| Weight | Accidental Deaths, per Cent | Sudden Meningococcic Deaths, per Cent |
|-------------|-----------------------------|---------------------------------------|
| Very light | 3.5 | 2.9 |
| Underweight | 10.5 | 16 |
| Normal | 23.0 | 17.4 |
| Overweight | 42.6 | 41 |
| Very heavy | 20.4 | 23 |

infection of the upper respiratory tract or a period of malaise. In 17 instances information regarding prodromal symptoms either was not available or had not been recorded. In none were the prodromal symptoms of sufficient severity to warn of impending catastrophe.

TABLE 13—*Duration of Prodromal Symptoms in Cases of Death from Meningococcic Infections*

| Duration | Number of Cases |
|------------------|-----------------|
| Less than 1 hour | 0 |
| 1-6 hours | 9 |
| 7-12 hours | 15 |
| 13-24 hours | 12 |
| 1-7 days | 28 |
| More than 1 week | 4 |

The duration of the prodromal disturbance in the instances in which a history was available was as shown in table 13.

Fulminant Phase—In most of the 95 cases in which death took place less than twenty-four hours after the onset of incapacitating illness, the transition from the prodromal to the fulminant phase of the infection

was abrupt and overwhelming. Seven of the men died before coming under medical observation, and 6 lived less than one hour after reaching the hospital. Sixty died within six hours after admission to a medical installation.

Because of the shocklike state of collapse associated with fever, cyanosis, cutaneous hemorrhages, rapid pulse, low blood pressure and early development of pulmonary edema, a large proportion of the cases were believed to be examples of Waterhouse-Friderichsen syndrome.⁸ In more than half of the group, Kernig's sign or painful or stiff neck was recognized at some time before death. In the majority of these, examination of cerebrospinal fluid disclosed meningococci, inflammatory exudate or both. Despite the frequency (about 50 per cent) with which signs and symptoms of meningocerebral involvement occurred, they were usually masked by those of peripheral circulatory failure. In a few instances manifestations of meningocerebral involvement were predominant throughout most of the brief illness.

Cutaneous hemorrhages were recognized clinically in 54 per cent of the group. The fact that a postmortem diagnosis of cutaneous hemorrhages appeared in 83 per cent of the autopsy protocols is consistent with the extremely rapid progression of the disease process.

In all instances in which body temperature was measured there was fever, and in more than half a reading of 40 C (103 F) or higher was obtained at some time before death.

Subnormal systolic and diastolic pressures were observed in almost every instance in which blood pressure was measured.

Pathologic Observations—In reviewing this group of sudden deaths, we found that 81 of the original 95 cases had sufficiently complete protocols and histologic material for a survey of pathologic changes. The four most frequently recurring pathologic diagnoses were cutaneous hemorrhage, adrenal hemorrhage, leptomeningitis and myocarditis. The frequency of these changes and of the combinations in which they occurred is shown in table 14.

Skin In 67 of 81 cases cutaneous hemorrhages were observed at autopsy. These varied in severity from a few scattered petechiae to large confluent purple patches over most of the body surface. In some the trunk was more extensively involved than the extremities, and in others the reverse was true. Frequently the distribution was described as "from head to toe." In many instances hemorrhagic lesions were also seen in the conjunctiva and in the buccal mucosa.

The microscopic appearance of the cutaneous lesions varied from simple interstitial extravasations of erythrocytes to hemorrhagic foci of

⁸ Waterhouse, R. *Lancet* 1:576, 1911. Friderichsen, C. *Jahrb f Kinderh* 87:109, 1918.

TABLE 14—*Relation of Adrenal Changes to Meningitis, Myocarditis and Cutaneous Hemorrhage*

| Categories of Adrenal Change | Cases | Meningeal Inflammation | | | Myocardial Exudation | | | Cutaneous Petechiae or Purpura | | |
|--|-------|---|---|--|--|---|----------------------|--|---|----------------------|
| | | Percentage of Total Cases of Adrenal Change | Percentage of Cases in Category Showing | | Cases in Category Showing Myocardial Exudation | Percentage of Cases in Category Showing | | Cases in Category Showing Petechiae or Purpura | Percentage of Cases in Category Showing | |
| | | | Cases in Inflammation | Per centage of All Cases of Meningeal Inflammation | | Myocardial Exudation | Myocardial Exudation | | Petechiae or Purpura | Petechiae or Purpura |
| Massive adrenal hemorrhage with complete cortical disorganization | 45 | 55 | 9 | 20 | 25 | 19 | 42 | 51 | 43 | 95 |
| Severe cortical degeneration and focal necrosis with extreme hyperemia and foci of hemorrhage | 13 | 16 | 7 | 54 | 10 | 8 | 62 | 22 | 11 | 83 |
| Extreme hyperemia and foci of hemorrhage | 4 | 5 | 2 | 50 | 6 | 3 | 75 | 8 | 4 | 100 |
| Minimal to advanced cortical degeneration and focal necrosis without hemorrhage or severe congestion | 14 | 17 | 14 | 100 | 39 | 4 | 29 | 11 | 5 | 36 |
| No significant abnormality | 5 | 6 | 4 | 80 | 11 | 3 | 60 | 8 | 4 | 80 |
| Total | 81 | 100 | 36 | 44 | 100 | 37 | 46 | 100 | 67 | 83 |
| | | | | | | | | | | 100 |

acute exudative inflammation Although bacteriologic studies of the cutaneous lesions were not made routinely, the impression was gained that meningococci were usually found in instances in which smears, cultures or appropriate stains of the cutaneous lesions were made

Adrenal Glands Because of the importance that various reviewers have attached to the adrenal changes in persons dying from fulminating meningococcemia and the difficulty that was encountered in attempting to evaluate from the autopsy protocols the degree and the kind of adrenal involvement present, it was decided to reexamine all slides of adrenal glands⁹

There were 81 cases in which at least one section stained with hematoxylin and eosin was immediately available Although it was realized that impressions derived from the examination of one or two slides were not necessarily valid for the total adrenal involvement, the results appeared to be of sufficient interest to warrant presentation

The cases appeared to fall into five principal categories so far as the adrenal involvement was concerned

- 1 No adrenal change In only 5 instances were the adrenal glands regarded as being essentially normal and without evidence of acute degenerative change, excessive hyperemia or hemorrhage
- 2 Cortical parenchymatous degeneration with little or no hyperemia or hemorrhage In 14 instances the adrenal glands, without significant capillary engorgement or hemorrhage, showed retrogressive changes in the cortical parenchyma of the kind characterized by Rich¹⁰ as "tubular degeneration"
- 3 Hyperemia without significant parenchymatous degeneration In 4 instances, there was severe hyperemia, most pronounced in the reticular zone and diminishing in severity as the capsule was approached In some of these, there were focal extravasations of erythrocytes In 58 instances the adrenal glands were grossly hemorrhagic
- 4 Focal hemorrhages with degeneration In 13 instances, the hemorrhages were focal, and surviving islands of cortex were the seat of striking tubular degeneration
- 5 Massive hemorrhagic necrosis In 45 instances of massive hemorrhage the accompanying cortical disorganization was so great that degenerative cellular changes were largely masked

Although the involvement was usually bilaterally symmetric, there were some instances in which the hemorrhage was massive in one adrenal gland and focal in the other

⁹ The complete presentation of the histologic changes seen in the adrenal glands in meningococcemia is beyond the scope of this paper, and will appear in a subsequent publication by one of us (N Z)

¹⁰ Rich, A R Bull Johns Hopkins Hosp 74 1, 1944

Although the precise sequence of changes that resulted in massive hemorrhagic necrosis of the adrenal glands could not be reconstructed from this study, certain inferences appeared to be justified. The impression was gained that the earliest change was a central hydropic degeneration within the cell masses of the glomerular and fascicular zones of the cortex. In this stage the newly formed central spaces contained only albuminous or fibrillar acidophilic material, later they contained polymorphonuclear leukocytes and debris of necrotic cells. This is the lesion characterized by Rich as tubular degeneration. In some instances the "tubules" may be distended with extravasated erythrocytes. The fact that this form of tubular degeneration without excessive hyperemia or hemorrhage was more commonly found than hemorrhage or excessive hyperemia without degeneration suggested that the parenchymatous lesion was probably primary. Although parenchymatous degeneration started in the glomerular zone and progressed inward, hyperemia characteristically started in the reticular zone and progressed outward. With increasing capillary engorgement, blood tended to escape into the central spaces of the injured cell columns. As the severity of the hemorrhage progressed, disorganization of the cortex occurred, and in its final stages only the reticulum and occasional epithelial cells served to locate the original position of the cortex in the massive perimedullary hematoma. In many instances adrenal glands that were the seat of massive hemorrhagic cortical necrosis were only slightly enlarged. In others they attained weights as great as 100 Gm.

Characteristically, the medulla was spared both hemorrhagic and degenerative change in all but the cases of most severe involvement. Significant antemortem thromboses were suggested in only a few instances.

Meningitis. In 36 of these 81 cases, acute leptomeningitis or meningoencephalitis was recognized. In the majority of these the reactive changes were relatively slight and were microscopic rather than macroscopic. In the instances in which the meningeal reaction was advanced, the changes were typical of acute meningococcic meningitis. It is noteworthy that severe meningeal involvement was rarely seen in association with massive hemorrhagic adrenal necrosis. A plausible inference is that a systemic infection of such severity as to produce bilateral hemorrhagic necrosis of the adrenal glands is likely to result in death before sufficient time has elapsed for the development of a full-blown meningeal reaction.

Myocarditis. No effort was made to appraise the severity or the functional significance of the myocardial involvement. It was apparent from the records that the diagnosis of myocarditis usually referred to an exudation of polymorphonuclear leukocytes overlying a monocytic reaction which was in some instances focal and in other instances diffuse and in some instances sparse and in others dense.

Other Pathologic Changes In addition, severe pulmonary edema and hyperemia were frequently observed. Petechiae were commonly found beneath the pleura, the epicardium and the endocardium. Acute splenic hyperplasia and cloudy swelling of the liver and the kidneys were noted. Focal hepatitis was recorded in several instances.

Relation of Adrenal Changes to Waterhouse-Friderichsen Syndrome
—The foregoing paragraphs have called attention to the fact that in 58, or 72 per cent, of these sudden deaths from meningococcemia post-mortem examination disclosed hemorrhagic lesions of the adrenal glands associated with acute retrogressive changes in the cortex. In the majority of these in which adequate anamnestic data were available the clinical course resembled that of the Waterhouse-Friderichsen syndrome. A typical example is as follows:

A 21 year old Japanese American was admitted to the hospital cyanotic and sleepy, complaining of nonproductive cough and slightly sore throat of two days' duration. Physical examination showed entirely normal conditions except for an injected nasopharynx and a temperature of 104.2 F. He was cooperative, answered questions clearly and undressed himself. Within five minutes of arrival in the ward he was obviously dying. The respiratory rate was 50, and the pulse was imperceptible. The heart sounds were obscured by rales. A purpuric rash was noted over the trunk and the extremities, and the patient died before any further diagnostic or therapeutic measures could be attempted.

At postmortem examination both adrenal glands were grossly hemorrhagic. The meninges were congested, but no exudate was present. Postmortem cultures of the blood were positive for meningococci.

Many of the soldiers whose course was that of the Waterhouse-Friderichsen syndrome also presented some clinical evidence of meningitis. These symptoms included confusion, coma, hyperexcitability and irrationality, delirium sometimes was so severe as to necessitate intravenous sedation. In the following case signs and symptoms of meningeal involvement were superimposed on the peripheral circulatory collapse of the Waterhouse-Friderichsen syndrome.

An 18 year old white soldier entered the dispensary with a chief complaint of nausea and vomiting of twelve hours' duration. The day before he had a sore throat and fever and remained in quarters. On physical examination, the temperature was 102.8 F, the respiratory rate was 32, the pulse rate was 132 and the blood pressure was obtainable. The patient appeared well oriented but nervous and apprehensive. There were generalized petechiae throughout the skin, and the pharynx was injected. There was moderate rigidity of the neck. The purpura increased rapidly, and within two and one half hours the temperature rose to 105.6 F, the pulse rate to 160 and the respiratory rate to 60. Within another hour he was delirious, and he died of respiratory failure four hours after admission. The white blood cell count was 14,400, with 72 per cent polymorphonuclear leukocytes. The clinical diagnosis was acute fulminating cerebrospinal meningitis.

At autopsy a small area of exudate was recognized on the superior surface of the right parietal lobe of the brain and was confirmed microscopically. There was massive hemorrhagic infiltration of the cortex of each adrenal gland.

There were other instances of extensive adrenal hemorrhage in which clinical evidence of meningeal involvement was predominant. An illustrative case of this kind is as follows.

A 20 year old Negro soldier was admitted to the hospital in coma. A history of headache and vomiting during the preceding night was obtained. On physical examination the patient was comatose and restless. The temperature was 104 F, the heart rate was 150 and blood pressure was 74 systolic and 50 diastolic. The pupils were small, and the eyeballs showed a slow rotary nystagmus. Pronounced rigidity of the neck and Kernig's and Babinski's signs were indicative of meningitis. The spinal fluid, however, was clear, with normal dynamics and without increased pressure. The peripheral white blood cell count was 2,850. Despite intensive therapy with sulfonamide drugs and penicillin, the patient went rapidly downhill. Two and one-half hours after admission the heart rate was 170, and the blood pressure was 50 systolic and 0 diastolic. He died three and one half hours after admission. At autopsy there were conjunctival petechiae. The adrenal glands were grossly hemorrhagic although not enlarged. Microscopically, the hemorrhage spared the medulla and was most intense in the zona reticularis of the cortex. Several cortical adenomas were also spared. The zona glomerulosa showed minor degenerative changes. The meninges showed no evidence of inflammatory change either grossly or microscopically. Cultures of blood and spinal fluid were positive for group 2A meningococci.

This case is of especial interest in that clinically it was considered one of meningitis and yet no laboratory evidence of meningitis was found either before or after death.

In 19 of the 81 cases in which a survey of pathologic changes was made, the adrenal glands presented neither gross nor microscopic evidence of hemorrhage. In 4 of these, microscopic examination disclosed moderately severe cortical changes of the "tubular degenerative" type. It is of interest that in all 4 of these cases the characteristic signs and symptoms of peripheral circulatory collapse were present. The following case is illustrative of this group.

An 18 year old white soldier was admitted to the hospital in a comatose condition. He had been found unconscious in a tourist camp, on physical examination, the temperature was 104 F and the respiratory rate was 62, the pulse rate and the blood pressure were unobtainable. The patient was moribund, cyanotic and gasping for breath rapidly and stertorously. No petechiae were seen. His pupils reacted only sluggishly to light. There was no nuchal rigidity. Bubbling rales and rhonchi were heard on auscultation over the entire chest. The white blood cell count was 33,600. On lumbar puncture 70 polymorphonuclear leukocytes were noted, and smear and culture of the cerebrospinal fluid revealed meningococci. Intensive fluid replacement and treatment with a sulfonamide compound and penicillin were instituted but produced no significant improvement, although the blood pressure rose within seven hours to 78 systolic and 58 diastolic. Respirations became progressively more rapid and shallow, and the patient died fifteen hours after admission. The diagnosis of Waterhouse-Friderichsen syndrome was made.

At autopsy there was pronounced cyanosis of the entire body. The adrenal glands, as well as all other viscera exhibited gross and microscopic congestion but

no hemorrhage Severe tubular degeneration of the adrenal cortex was present There was early meningitis, with mononuclear as well as polymorphonuclear leukocytes in the subarachnoid space

In 10 of the 19 cases in which the adrenal glands were nonhemorrhagic the degenerative changes in the cortex were characterized as minimal Central liquefaction of cell masses was confined to the glomerular zone and the outer portion of the fascicular zone All 10 of the patients clinically and pathologically evidenced meningitis Clinical manifestations of peripheral circulatory collapse were present in some of them

In 5 of the cases in which the adrenal glands were nonhemorrhagic, the cortical zones showed no evidence of acute retrogressive change In 4 of these, postmortem examination disclosed advanced purulent leptomeningitis An example of this group is as follows

An 18 year old white soldier was admitted to the hospital in a comatose condition The temperature was 104.2 F, the pulse rate was 88 and the respiratory rate was 22 On physical examination, stiff neck, Kernig's sign and twitching of lips, fingers, toes and arms were evidence of cerebral irritation, but there were no generalized convulsions Lumbar puncture revealed cloudy fluid containing 14,400 leukocytes, 92 per cent of which were polymorphonuclear leukocytes Smear and cultures were positive for meningococci Sulfadiazine was administered intravenously, but the patient sank more deeply into coma and died two and one-half hours after admission

At postmortem examination, there were no cutaneous petechiae or purpura, and the adrenal glands were grossly and microscopically normal There were numerous perivascular hemorrhages extending into the cerebral parenchyma Profound edema was noted in both cortical and subcortical tissue In some sections the meninges contained a heavy exudate of polymorphonuclear leukocytes, and there was also a considerable leukocytic infiltration of the superficial layers of the molecular layer of the cerebellum

Clinically Obscure Deaths Due to Meningococcemia—In general, the correctness of the clinical evaluation of these cases was proportional to the duration of clinical observation In the majority the duration and the character of the terminal illness were such as to warrant a presumptive or a positive antemortem diagnosis of meningococcic infection There were many instances in which the illness was so brief and overwhelming that its true nature was not suspected prior to autopsy The following cases are examples

At 6 p. m. a 19 year old white soldier complained to the charge of quarters of headache and of "feeling wobbly" When asked whether he wished to go on sick call, he stated that he would wait until morning Three hours later he again complained of headache, and at 1 a. m. he was heard groaning At 5.45 a. m. he was found dead in bed Postmortem examination revealed massive adrenal hemorrhage without meningitis

A 20 year old white soldier was admitted to an evacuation hospital without previous known history He appeared restless and somewhat drowsy and was sent

to the medical ward for diagnosis. Thirty-five minutes later he was found dead. At autopsy, discrete and confluent hemorrhages were found throughout the cutaneous surface. The adrenal glands were enlarged, and both cortex and medulla were entirely obscured by massive hemorrhage. Although edema and congestion of cerebral and meningeal vessels were found, there was no microscopic evidence of meningitis.

Sudden Death and the Waterhouse-Friderichsen Syndrome—Most published reviews dealing with the subject of sudden death give little or no emphasis to meningococcemia as one of the causes. References to rapidly fatal meningococcal infection occur in reviews by Spilsbury¹¹ and Simpson¹². Whenever meningococcemia is discussed as a cause of sudden death, the Waterhouse-Friderichsen syndrome is usually described, and death is attributed to the massive adrenal hemorrhage. Although there are many reported cases of sudden death associated with massive adrenal hemorrhage in children, the syndrome seems to have been remarkably rare in adults. Recent reviews treating of the Waterhouse-Friderichsen syndrome include a total of fewer than 30 cases in which the patient was an adult. For this reason the frequency with which massive adrenal hemorrhage was found in soldiers with rapidly fatal meningococcal infection was not anticipated. In approximately 209 of 352 deaths among soldiers due to meningococcal infection, there was some degree of hemorrhage in the adrenal glands. No such incidence of hemorrhage in these glands appears in reports of examinations of autopsy material from civilian sources.

There are several possible explanations of this apparent discrepancy. One is that in many cases of acute fulminating meningococcal infection death takes place early in the disease. As we have stated, in 110 of 352 cases of fatal infection, death occurred in the first twenty-four hours of illness. Many patients were found dead or died within a few hours after the onset of severe illness. In civilian life most of these would have become coroner's subjects, with the result that little or no reliable information concerning the postmortem observations would have been obtained. That the rarity of the Waterhouse-Friderichsen syndrome among civilian adults is apparent rather than real is indicated in a recent report by Dr. Harrison Martland,¹³ medical examiner of Essex County, N. J., who collected 9 cases. His series of autopsies following deaths of adults from massive adrenal hemorrhage is the largest described by a single investigator.

The high incidence of sudden death from this cause among soldiers is further explained by the fact that meningococcemia is an epidemic

11 Spilsbury, B., in Rolleston, H. *The British Encyclopaedia of Medical Practice*, London, Butterworth & Co., Ltd., 1937, vol. 3, p. 565.

12 Simpson, C. K. *Lancet* **1** 851, 1937.

13 Martland, H. S. *Arch. Path.* **37** 147, 1944.

disease which is particularly likely to appear when large groups of people are brought together, as in military mobilization

MISCELLANEOUS CAUSES OF UNEXPECTED DEATH

Early in this investigation the idea of making a statistically significant study of all causes of sudden death of young soldiers was abandoned. Although many deaths from a wide variety of causes had been coded as "sudden," exploration of various other categories of postmortem diagnoses disclosed that death occurring within twenty-four hours after the patient came under medical observation or after the onset of incapacitating symptoms was not always designated "sudden death." To have collected all sudden deaths from every cause would have required individual examination of almost every one of the more than 40,000 autopsy protocols received from 1942 to 1946.

It was apparent from random sampling that a multiplicity of potentially fatal diseases was responsible for unexpected deaths. In many instances it was difficult to understand how a person could have carried on until so short a time before collapse and yet have had so little evidence of illness that he had neither reported for sick call nor appeared to be ill.

Attention has already been called to the fact that the majority of such deaths resulted from organic heart disease, intracranial hemorrhage, meningococcemia or causes not disclosed by postmortem examination. Other occasional and less frequent causes of sudden death are as follows:

- | | |
|-------------------------------|--|
| 1 Adrenal atrophy | 8 Hernia, intestinal obstruction |
| 2 Aortic rupture | 9 Meningitis (other than meningococcie) |
| Syphilis | 10 Pancreatitis, acute hemorrhagic |
| Arteriosclerosis | 11 Pharyngolaryngitis (obstructive) |
| Idiopathic medial necrosis | 12 Pneumonia |
| 3 Diabetes mellitus | Bronchopneumonia (streptococcie) |
| 4 Diphtheria | Lobar pneumonia |
| 5 Encephalitis | 13 Septicemia (other than meningococcemia) |
| 6 Glioma (without hemorrhage) | 14 Thrombophlebitis (pulmonary embolism) |
| 7 Hepatitis, acute infectious | 15 Tuberculosis, pulmonary |
| (Acute yellow atrophy) | |

Although no effort was made to determine the total number of unexpected deaths from each of these miscellaneous causes, it was apparent that the largest number was due to bronchiopneumonia. In this group it was the exception rather than the rule when death was reported to have occurred within a few minutes after the onset of the fatal seizure. Usually there was an interval of a few hours or more between collapse and death. On the other hand, many soldiers with this disease were found dead.

CAUSE OF DEATH NOT DISCLOSED BY AUTOPSY

In addition to the deaths from definitely recognized diseases and a large unclassified group excluded because of inadequate clinical, pathologic or toxicologic data, there were at least 140 carefully investigated

cases in which the postmortem findings were essentially normal. Not only did the protocols of these cases indicate that a complete autopsy had been performed, but in most instances the histologic preparations had been reviewed by two pathologists, one at the referring laboratory and the other at the Army Institute of Pathology. Cases in which the postmortem findings were of questionable significance were not included. Thus a death in which the only postmortem finding of consequence was "moderately severe but nonocclusive coronary arteriosclerosis" was rejected. The death could not be classified as one in which the postmortem findings were normal, nor could it be categorically attributed to coronary disease. On the other hand, 16 cases in which the postmortem examination was noncontributory except for "occasional small intimal plaques in the coronary arteries" were classified as "cause of death not disclosed by autopsy."

TABLE 15—*Age Distribution in Cases of Sudden Death from Causes Not Disclosed at Autopsy (135 Cases*)*

| Age, Years | Unexpected Deaths from Obscure Causes, per Cent | Total Army Personnel, per Cent |
|------------|---|--------------------------------------|
| Under 19 | 3 | 9 |
| 20-24 | 36 | 38 |
| 25-29 | 31 | 30 |
| 30-34 | 15 | 16 |
| 35-39 | 15 | 6 |

* In 5 instances the exact age was not given, the soldier being described as young.

Also excluded from this group of deaths from unknown causes were those in which there was a reasonable chance that death might have resulted from the ingestion of some unrecognized poison (including alcohol), from anaphylactic shock or serum sickness following a recent inoculation or injection, from heat stroke, from high altitude anoxia or from trauma.

Age—The ages of the soldiers comprising this group are shown in table 15.

Between the ages of 20 and 35 the number of deaths in each group is in direct ratio to the total number of soldiers in that age range in the Army. It may be seen, however, that soldiers under 20 appeared to be less and those over 35 more likely to die suddenly of undisclosed causes than were men between 20 and 35 years.

Race—The ratio of Negroes to white persons was 15:125, which is in fair agreement with the ratio of Negroes to white persons in the Army.

Obesity—The relation of body weight to the unexpected death of 100 soldiers from obscure causes is given in table 16.

There does not appear to be any significant relationship between body weight and death from anatomically obscure causes

Exercise—The records of 127 cases were reasonably explicit as to what the soldier was doing at the time of or immediately preceding the fatal seizure. These observations are summarized in table 17

TABLE 16—*Weight Distribution in Cases of Sudden Death from Obscure Causes (100 Cases)*

| Weight | Sudden Deaths from Obscure Causes, per Cent | Accidental Deaths, per Cent |
|------------------------|---|-----------------------------------|
| Most underweight | 1 | 3.5 |
| Moderately underweight | 9 | 10.5 |
| Normal | 24 | 23.0 |
| Moderately overweight | 43 | 42.6 |
| Most overweight | 23 | 20.4 |

It does not appear in this series that there was any relationship between physical activity and the onset of the fatal episode. The fact that the collapse occurred incident to or immediately after strenuous physical exertion in 23 per cent of the cases indicates that at least this

TABLE 17—*Relation of Type of Activity to Sudden Death from Obscure Causes (127 Cases)*

| Type of Activity | Estimated Time Spent in Inactivity and Activity, per Cent | Fatal Seizures Coincident with Inactivity or Activity, per Cent |
|--------------------------|--|--|
| Sleep | 33 | 24 |
| Inactivity—soldier awake | 17 | 23 |
| Ordinary activity | 33 | 31 |
| Strenuous exertion | 17 | 23 |

number of soldiers probably felt reasonably well until a short time before collapse and death. That the fatal collapse of these apparently healthy persons was not invariably a vasomotor phenomenon activated by some extrinsic stimulus was indicated by the fact that in about

TABLE 18—*Survival Time After Onset of Fatal Seizure*

| Time | Cases |
|--|-------|
| Few minutes or less | 93 |
| More than a few minutes and less than 60 minutes | 4 |
| Between 1 and 24 hours | 4 |
| Not known or not recorded | 39 |

24 per cent of them the fatal seizure occurred during sleep or immediately after waking

Survival Time After Onset of Fatal Seizure—The rapidity with which death occurred after the onset of incapacitating symptoms is shown in table 18

State of Health Immediately Preceding the Fatal Seizure—The information recorded with regard to the state of health during the twenty-four hours that preceded collapse is given in table 19

TABLE 19—*State of Health Preceding Collapse*

| State of Health | Cases |
|--|-------|
| "Did not feel well" | 10 |
| Substernal or epigastric discomfort | 9 |
| Acute psychotic episode with extreme agitation | 8 |
| Headache | 7 |
| Infection of the upper respiratory tract | 3 |
| Vomiting | 3 |
| Normal or not stated | 100 |

TABLE 20—*Characteristics of Fatal Seizure*

| Observation | Cases |
|---|-------|
| Simple syncope (rarely preceded by vomiting or followed by labored respiration) | 68 |
| Convulsions | 17 |
| Substernal or epigastric pain and syncope | 9 |
| Headache and syncope | 2 |
| Not known or not recorded | 44 |

Nature of the Fatal Seizure—In 96 instances, the attack was witnessed, and its characteristics were recorded. These are summarized in table 20

Past History—In the majority of these cases, either there was no comment regarding the previous health or it was reported as having

TABLE 21—*Postmortem Findings in 140 Cases of Unexpected Death from Obscure Cause*

| Findings | Cases |
|--|-------|
| Agonal changes | |
| Cardiac dilatation | 13 |
| Pulmonary edema and congestion | 69 |
| Generalized congestion of viscera | 19 |
| Disseminated petechiae | 12 |
| Cerebral petechiae | 4 |
| Evidence of disease | |
| Obesity | 23 |
| Cardiac hypertrophy (over 400 Gm.) | 18 |
| Coronary arteriosclerosis (occasional small atheroma) | 16 |
| Focal myocarditis (mild and of doubtful significance) | 3 |
| Fat infiltration of right ventricle | 2 |
| Craniocerebral adhesions | 2 |
| Purulent bronchitis | 1 |
| Chronic catarrhal bronchitis (chronic asthma?) | 1 |
| Interstitial pneumonitis (mild and of doubtful significance) | 1 |
| Generalized arteriolar sclerosis | 1 |
| No pathologic change | 33 |

been good. In 10 instances a history of previous convulsive seizures suggested epilepsy.

Pathologic Observations—The postmortem findings in this group are summarized in table 21.

It is apparent that the most frequent postmortem changes noted in this group are classifiable as agonal changes and are probably indicative of acute circulatory failure or acute systemic anoxia. Certainly whatever evidence there was of disease was not adequate to explain the unexpected failure of circulation or of respiration which was the immediate cause of death. The most frequently encountered evidence of disease was cardiac hypertrophy. In only 1 instance, however, did the weight of the heart exceed 450 Gm. In this case the cardiac hypertrophy was accompanied by diffuse vascular disease.

There is little to be said in summary of the pathologic observations other than to reiterate that the methods of examination available to the pathologist (or the toxicologist) are frequently inadequate to disclose either the extent or the nature of disorders even though they are of sufficient severity to be incompatible with life.

SUMMARIZING COMMENT

From an incomplete survey of the autopsy material at the Army Institute of Pathology it was estimated that reports of approximately 1,000 sudden deaths from disease of apparently healthy soldiers between 18 and 40 years were received between 1942 and 1946. Although an attempt to review all of them was not made, an examination of between 700 and 800 indicated five principal categories.

Heart Disease—There were approximately 350 sudden deaths from previously unrecognized heart disease. Almost 300 of these were due to coronary arteriosclerosis. The following facts and opinions were derived from an analysis of the data on 115 soldiers whose deaths were from coronary disease.

Eight per cent of the group were under 25 years of age, and 22 per cent were younger than 30. White and Negro soldiers were represented in proportion to their numbers in the Army.

Although the body weights of these soldiers as recorded in the autopsy protocols were significantly greater than those recorded for healthy inductees, this was equally true of the weights recorded in the autopsy protocols of soldiers dead of accidental injuries from firearms, traffic accidents and acute infections. It was concluded that the figures for body weights in these autopsy protocols were usually estimates and should be used with reservation to judge the relationship of obesity to any given disease.

The frequency with which the onset of the fatal attack of coronary insufficiency occurred during a period of strenuous physical exertion lends support to the prevalent and plausible opinion that violent exercise is probably dangerous for persons suffering from severe coronary disease. That this information is not of great practical value to the Army in the prevention of such casualties is indicated by the fact that none

of these soldiers was suspected of having heart disease prior to death and that even in retrospect fewer than 25 per cent of them had a history of symptoms that may have been of cardiac origin

The practicability of conducting complete cardiologic studies on all soldiers who admit having occasional twinges of abdominal or epigastric discomfort or of relegating them to a limited service status is dubious to say the least

In all instances postmortem examination disclosed severe atherosclerosis of one or both coronary arteries. Thrombotic occlusion was recognized in approximately 25 per cent, and it was apparent that in most of these the fatal thrombus had begun to form some hours or days before it became symptomatic. In an additional 20 per cent the occlusion was attributed to atheromatous change. Bleeding into an atheromatous plaque was an infrequent cause of occlusion. In 55 per cent of the group no site of complete occlusion was found

Intracranial Hemorrhage—In 69 of 91 reviewed cases of sudden death from nontraumatic intracranial hemorrhage the bleeding was principally subarachnoid and was either proved or inferred to have resulted from the rupture of a superficial aneurysm of the congenital or berry type

The frequency of sudden death from this cause tended to increase with age between 18 and 40 years. The incidence was similar in white and Negro soldiers. There was some indication that aneurysms of this type are more likely to rupture during a period of violent physical exertion than during sleep. About 20 per cent of these soldiers gave a history of headaches

At autopsy the bleeding was found to be confined to the subarachnoid space in about 65 per cent and was both parenchymatous and subarachnoid in 35 per cent. The latter type of hemorrhage was usually due to the rupture of an aneurysm which had become attached to or had burrowed into the adjacent cortex. Although many soldiers died during their first episode of hemorrhage, others showed postmortem evidence of previous bleeding that had not been recognized clinically

Meningococcemia—In 110, or approximately one third, of all reported cases of death from meningococcal infections, death occurred within twenty-four hours after the onset of incapacitating symptoms. The incidence of fatal meningococcemia bore an inverse relationship to age and was higher in Negro than it was in white troops

More than half of the patients in this group died within six hours after coming under medical observation. In approximately 70 per cent of the cases, the soldier was reported to have been below par or to have been suffering from a mild infection of the upper respiratory tract prior to the onset of the fulminating phase of the disease. It was

apparent that no "cold" or minor indisposition complained of by a soldier who may have been exposed to persons with meningococcic infections should be ignored. The majority of those who died after a fulminant phase of twenty-four hours or less had unheeded warning of the impending catastrophe.

Many were found dead or died before there was opportunity for clinical study. In a few instances, the signs and symptoms were predominantly those of meningitis, and circulatory failure was terminal. The majority, however, were characterized clinically as suffering from the Waterhouse-Friderichsen syndrome, some with and many without evidence of meningeal invasion.

At autopsy purpura or cutaneous petechiae were recognized in over 80 per cent of these men. Focal or massive adrenal hemorrhages were encountered in 71 per cent. In most of the hemorrhagic and the nonhemorrhagic adrenal glands, microscopic examination of the cortex disclosed degeneration and necrosis of the "tubular" type.

Although there was no instance in which the Waterhouse-Friderichsen syndrome was reported to have been observed in a person whose adrenal glands were found to be normal, adrenal hemorrhage was not prerequisite to the occurrence of a shocklike state of collapse nor was it invariably associated with bleeding into the skin. Adrenal apoplexy occurred without cutaneous hemorrhage, and cutaneous purpura was encountered without hemorrhage of the adrenal glands.

The impression was gained that adrenal hemorrhage in fulminating meningococcemia was usually preceded by degenerative changes in the cells of the glomerular and fascicular zones of the cortex. Medullary damage was characteristically absent, minimal or terminal. Gross or microscopic evidence of meningitis was present in 44 per cent and of myocarditis in 46 per cent of all cases.

Miscellaneous Causes of Sudden Death—Although inflammatory, degenerative and neoplastic diseases of wide variety were encountered as occasional causes of sudden death, the number of deaths resulting from any one of them was not of the same order of magnitude as those of the three previous groups. The impression was gained that the total number of reported sudden deaths from miscellaneous causes was probably between 200 and 400 and that infections of the respiratory tract accounted for the largest subgroup.

Obscure Causes—There were at least 140 carefully investigated sudden deaths in which the postmortem findings were essentially normal. Complete pathologic examination, supplemented in many instances by toxicologic studies, failed to disclose the cause of death. It was estimated that they represented more than 10 per cent of all sudden non-traumatic deaths of apparently healthy young men.

The racial and the age distribution of the soldiers dying of obscure causes corresponded to those of the Army as a whole. Their weights did not differ from those of soldiers dead of accidental violence or of acute infections. The majority either were found dead or died within a few minutes after a syncopal attack.

In 8 instances death was preceded by an acute psychotic disturbance manifested by violent emotional and physical agitation. In 10 instances there was a past history of convulsive seizures suggestive of epilepsy.

There was no apparent relationship between the onset of the fatal seizure and what the soldier was doing at the time of the attack. Thus, the number of seizures that occurred during sleep or during strenuous exertion was roughly proportional to the number of hours that the average soldier devoted to such activities.

There is little to be said in summary of the pathologic observations of this group other than to reiterate that the methods of examination available to the pathologist are frequently inadequate to disclose either the extent or the nature of certain disorders even though they are of sufficient severity to be incompatible with life.

FINAL SUMMARY

Survey of the postmortem material at the Army Institute of Pathology revealed that the most common diseases responsible for rapid, unexpected death among young soldiers were heart disease, intracranial hemorrhage and meningococcemia. Of the many miscellaneous diseases also recorded, pneumonia occurred most frequently. In addition there was a group of deaths for which no cause could be established.

The most common form of heart disease responsible for sudden death was severe coronary arteriosclerosis with or without demonstrable thrombosis. Rupture of an aneurysm of the circle of Willis was the most common cause of rapidly fatal intracranial hemorrhage. Degeneration of and hemorrhage into the adrenal cortex were regarded as important contributing causes of rapid collapse and early death incident to meningococcemia.

CARDIOAORTITIS

Report of a Case

NIELS DUNGAL

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INFLAMMATIONS of the aorta are commonly considered to be traceable to one of two causes syphilis and rheumatic fever Jores¹ maintained that a productive inflammation of the aorta may practically always be traced to syphilis. In European institutes it has perhaps been taken far too much for granted that all kinds of productive aortitis are of this origin and closer inquiries neglected for that reason.

Klotz² described inflammation of the aorta from a nonsyphilitic cause in 1915 and Von Glahn and Pappenheimer³ described arteritis of rheumatic origin in 1926. In all countries the sporadic occurrence of periarteritis nodosa has fanned the interest in arterial inflammations of nonsyphilitic origin, leading to the important observations of Rich that those lesions are traceable to anaphylactic hypersensitivity.

The case to be described here may fall into this group, although its final cause remains unexplained.

REPORT OF CASE

April 3, 1940 a man 22 years old was received in the medical department of the state hospital in Reykjavik. He came from the country, where he had lived all his life, having but little communication with the outside world. His father had died of heart disease, and his mother had, in her youth, recovered from tuberculosis. The patient was in good health until the age of 12 years, when he began to have pains in various joints with fever, which sometimes rose to 40 C (104 F). These attacks recurred again and again but only rarely lasted longer than a few days. In the summer of 1936, exposure to cold was followed by lasting fever, and he lay in a provincial hospital with the diagnosis of his condition uncertain. There was no expectoration during this fever, but the patient had swollen glands on both sides of his neck. Some of these glands were incised, apparently without evacuation of pus, and after a month the wounds were healed.

In May the patient suffered from severe pains in the head and on that account lay in bed for a month at home. He was then thought to have meningitis, but recovered.

In April 1938 the patient's health got worse, and all the time he had enlarged glands in the neck.

From the Department of Pathology, University of Iceland

1 Jores, L., in Henke, F., and Lubarsch, O. *Handbuch der speziellen pathologischen Anatomie*, Berlin, Julius Springer, 1924, vol 2, p 667

2 Klotz J M *Research* 31 409, 1915

3 Von Glahn, W C, and Pappenheimer, A W *Am J Path* 2 235, 1926

In the winter of 1940 he had an attack of influenza, and his glands enlarged once more. His strength gave away, but he had no pains and no expectoration. When the patient was hospitalized, his right pupil was considerably wider than the left, and he had only 50 per cent vision with the left eye. Under the right mandibular angle were enlarged glands of cherry size, and similar but less enlarged glands were palpable on the left side. There were no enlarged glands in the axillae.

Ophthalmoscopy revealed old adhesions between the iris and lens.

On April 30 the patient had a chill and complained of pains in both ankle joints, while the fever rose to 39 C (102.2 F). These attacks were repeated several times until the patient died, May 23. During the last days of life he had convulsions, and the blood urea rose to 200 mg per hundred cubic centimeters.

Observations made at the hospital were as follows. The blood pressure was 125 systolic and 90 diastolic. The hemoglobin was 73 per cent (Sahli). The erythrocyte sedimentation rate was 125 mm in one hour. The urine contained albumin and pus but no sugar. By Esbach's reagent the albumin in the urine was 10 per cent. The erythrocyte count was 4,320,000, the leukocyte count was 3,228, of which 22 per cent were segmented and 48 per cent unsegmented neutrophils, with lymphocytes 30 per cent.

Autopsy—The body was 185 cm long and not emaciated. There was slight edema around the ankles.

No adhesions were noted in the thoracic or the abdominal cavity.

The heart weighed 520 Gm and was smooth on the surface. No fibrosis was visible in its musculature, and the endocardium was smooth. Behind the semilunar valves the lining of the aorta was distinctly changed, a finely wrinkled sack was seen behind each valve, yet the sacks did not resemble aneurysms, as the distention was so diffuse that its limits were hardly discernible, but the size of each sack corresponded to that of a grape. The valves themselves were smooth and thin, with no macroscopic changes.

The aorta had no apparent changes in the ascending part, but in the descending part of the thoracic portion changes were seen similar to those at the base, i. e., wrinkled, slightly bulging patches where the intercostal arteries branched from the aorta. These wrinkled patches were about the size of a dime, all soft and uncalcified.

Below the diaphragm these changes were much more conspicuous. A slight dilatation was noticeable where the superior mesenteric artery branched from the aorta, and from there downward the aorta in its entirety was more or less thickened and wrinkled, with light brown patches. When cut, the tissue was soft, white and without any resemblance to lipid material, and no calcifications were found. The whole wall was soft and appeared to be less elastic than normally.

The lungs showed no abnormality. In both hilar regions enlarged lymphatic glands were seen, especially on the right side, where chalklike caseations were present in some glands, and on the left side one gland was found thoroughly calcified. Caseated glands were found extending along the veins of the neck on both sides.

The liver was greatly enlarged, weighing 2,700 Gm, and was of rather increased consistence. Its surface was smooth and brownish, the cut surface appeared fairly normal.

The spleen weighed 700 Gm and was of medium consistence and dark red on the cut surface.

Both kidneys were greatly enlarged, each weighing 320 Gm. They were of the same appearance—the surface, light gray, the capsule, easily detached, the parenchyma, light gray, without distinct limits between pyramids and cortex.

Nothing conspicuously abnormal was seen in the intestinal tract.

The tonsils were not enlarged. The thyroid gland weighed 27 Gm.

The brain was unusually heavy, weighing 1,740 Gm, but was without apparent abnormalities. The hypophysis weighed 0.68 Gm, and was apparently normal.

Blood Cultures—Blood agar and ordinary mediums were inoculated from the blood of the heart. No growth was obtained.

Kahn Test—The Kahn test was made on the blood while the patient was alive and also after his death, but with negative results.

Histologic Examination—(a) *Liver* There was conspicuous hyperemia, and the central venules were considerably dilated. The hepatic cells were fairly well outlined, but the trabecular structure was partly destroyed by the distended capillaries.

(b) *Aorta* The aortic wall was greatly thickened, chiefly from hyaline fibrous tissue. This increase in thickness was mainly due to thickening of the intima, which was hyaline, but in some places it contained smaller or larger cell infiltrations, consisting mostly of neutrophil leukocytes, endothelioid cells and histiocytes scattered in a necrotic tissue where nuclear destruction was prominent among small and partly larger hemorrhages. Small infiltrates of leukocytes were seen scattered in many places, particularly around vessels, some of which were fairly wide in the media, which in its entirety was greatly thickened by fibrosis. Hyaline necrosis was seen in some places in the connective tissue, and in between these foci smaller and larger fluid-filled spaces were seen, as if colliquative necrosis had taken place. Some of these spaces contained a number of large cells with small nuclei and large amounts of protoplasm, which would be only faintly stained, giving the appearance of water-logged protoplasm. Similar cells could also be seen scattered in the intima. Many clefts were seen in the tissue, some filled with blood, but others not. In the vasa vasorum no intimal changes could be seen, but around some of them were small heaps of lymphocytes. In the connective tissue a scattered infiltration of segmented leukocytes was taking place, if no heaps of them were present. Of the cells surrounding the vasa vasorum, a considerable proportion consisted of plasma cells. No micro-organisms were found.

(c) *Heart* A quick survey showed little change in the heart, but on closer search scattered small accumulations of segmented leukocytes and plasma cells were found, most of them slight. In one such accumulation, which was among the largest and consisted chiefly of histiocytes, a structureless substance was seen between the cells, which appeared to consist of fibrin. Most of the cells were lymphocytes and monocytes, with a few plasma cells and fewer endothelioid cells. In some places fibrotic patches were noted, with some remnants of muscular tissue still discernible.

(d) *Kidneys* The glomerular capillaries were for the most part hyalinized, and in some of them no cells could be seen, but only a structureless substance which retained the form of capillary tufts. Staining with gentian violet displayed much amyloid in the malpighian corpuscles and in some places in the convoluted tubules.

COMMENT

The disease described here does not to my knowledge conform with any previously described. The macroscopic and microscopic

changes were different from those seen in rheumatic involvements. And in recurrent rheumatic disease endocardial changes would be expected, which were not present in this case. Aschoff's nodules were not found, and the aortic changes were prominent in the intima and the media, instead of being limited to the adventitia as they usually are in rheumatic infections. The pericardium had never been involved, the pathologic process being limited to the muscular tissue of the heart.

Syphilis can be excluded with certainty. Not only were the blood tests negative, but the aortic changes were most pronounced in the abdominal portion, not in the thoracic part as is the rule in syphilis. Besides this man was far too young for mesoarteritis to have had time to develop, and there is no reason to suspect syphilis in a man who had stayed all his life in an out of the way rural community where syphilis is unknown.

We can therefore with great probability exclude a syphilitic as well as a rheumatic infection as the cause of this disease.

The patient had a tuberculous involvement of the lymphatic glands of his neck. This was microscopically found to be a typical caseous tuberculous process which was in no way similar to the changes found in the heart and the aorta.

The patient appears, then, to have been suffering from two diseases at the same time: tuberculous infection of the cervical lymph glands and a different process involving his vascular system, particularly pronounced in the heart and the aorta but manifesting itself also in the amyloid nephrosis of the kidneys. The enlargement of the spleen was partially due to amyloidosis but probably only partially, as it is unusual to see such great enlargement of the organ from amyloidosis alone.

The changes in the liver were noteworthy, as there was excessive hyperemia without any definite signs of venous stasis.

Culture mediums inoculated from heart blood and spleen remained sterile and one is therefore not justified in assuming a streptococcal origin of the infection.

I have not been able to find any report of a corresponding case in the literature. In typhus and scrub typhus, according to Allen and Spitz,⁴ similar changes may be observed in the aorta, although in a lesser degree, but a typhic origin can be excluded in this case.

Klotz² has published a case of aortitis in which the inflammation was limited to the places in the aortic wall where the intercostal arteries branch out from it. The changes in his case seem to have been similar to those described here.

4 Allen, C. A., and Spitz, S. *Am J Path* 21: 603, 1945.

Chiari⁵ and Marchand⁶ have suggested that a smaller or greater number of the cases classified as instances of aortitis of syphilitic origin may have nothing to do with syphilis

Sproul and Hawthorne⁷ have described a case of chronic diffuse mesaortitis which had no relation to syphilis. But the patients were old patients. The aorta was macroscopically unchanged, and no symptoms of infection were mentioned in the anamnesis. The aortitis described here is therefore apparently different.

A case similar to mine has been described by Mallory⁸

The patient was a 35 year old man. Four years before death he began to suffer from pains in his feet, with signs of inflammation in the metatarsal joints. A year later he was troubled by pains in his chest, and at intervals he had pains in various joints, but never acute arthritis.

Behind the aortic valves and in the initial 25 cm of the ascending aorta was a pannus-like thickening of wrinkled fibrotic pink tissue, but no atheromatosis was visible. All valves were intact, and nowhere was there a sign of rheumatic involvement. The heart was greatly hypertrophied, weighing 900 Gm.

Microscopically, the connective tissue was greatly increased, with slight, if any, inflammatory reaction. Accumulations of lymphocytes were found along the vasa vasorum and considerable perivascular infiltration of the adventitia was noted, forming a striking resemblance to acute syphilis.

The same author mentioned another case.

A 25 year old man six years before had fallen ill with swellings of the joints of his feet and had suffered from that affliction for two years. Later his carpal joints were affected, and then those of the spinal column, but yet he kept on working. Some time later all his arthritic inflammations flared up, and his condition led to death.

At autopsy the heart was enlarged, weighing 580 Gm., and much the same changes were noted in the aorta as in the case previously described, but the changes reached only 1 cm into the aorta. On the other hand, the process reached onto the endocardium in the ventricle below the semilunar valves. Microscopically the picture was similar except that the infiltrate of leukocytes, monocytes and lymphocytes was more pronounced. Mallory⁸ found these changes greatly resembling acute syphilis. In the heart the muscular tissue was found degenerated with fibrosis.

Mallory said that most pathologists would have classified his cases as instances of syphilis but that, although he was unable to prove that the changes described were not of syphilitic origin, he must have serious

5 Chiari, H. Verhändl d deutsch path Gesellsch **15** 137, 1904, cited by Jores¹

6 Marchand, F. Verhändl d deutsch path Gesellsch **15** 197, 1904, cited by Jores¹

7 Sproul, E. E., and Hawthorne, J. J. Am J Path **13** 311, 1937

8 Subacute Aortitis and Aortic Endocarditis, Etiology Unknown, Cabot Case 22141, New England J Med **214** 690, 1936

doubts in that respect. He suggested that his cases may be instances of a disease of some unknown origin, perhaps a disease to be considered as precursory to the inexplicable calcifications in the cardiac valves of old people, which as a rule are not accompanied by endocarditis.

Holman⁹ in a series of papers has shown that acute arteritis can be produced in dogs if they are fed a low protein diet and at the same time suffer from renal insufficiency.

The excessive amyloid nephrosis in the present case without corresponding amyloidosis of other organs may have been a causative agent in producing the acute aortitis. This amyloid nephrosis in all probability caused hypoproteinemia, although I have no proof of that condition, as serum protein determinations were not made.

Rich and Gregory¹⁰ have shown experimentally that lesions with the basic characteristics of rheumatic carditis can be the result of anaphylactic hypersensitivity.

Rich¹¹ has demonstrated the causal relationship between allergic hypersensitivity and periarteritis nodosa. Holman⁹ mentioned also the apparent connection between renal insufficiency and periarteritis nodosa.

The unusually outspoken vascular changes in my case might be the result of hypersensitization following or concomitant with the renal lesion, although the mechanism remains obscure. Although this patient had a tuberculous infection which could have furnished the allergen, other antigenic agents might have been responsible.

SUMMARY

Severe cardioaortitis occurred in a 22 year old man. Syphilis could be excluded, and the clinical and pathologic picture was not that of a rheumatic infection. It is suggested that allergic hypersensitivity may have been the basic causative factor.

9 Holman, R. L. *Am J Path* **19** 147 and 159, 1943.

10 Rich, A. R., and Gregory, J. E. *Bull Johns Hopkins Hosp* **73** 239, 1943.

11 Rich, A. R. *Bull Johns Hopkins Hosp* **71** 123 and 375, 1942.

DISTRIBUTION OF ALKALINE PHOSPHATASE IN THE HUMAN LIVER

A Study of Postmortem Material

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IN PREVIOUS reports we¹ described the morphologic distribution of the alkaline phosphatase of the liver as observed under various experimental conditions. In the present investigation the distribution of the enzyme was studied in tissue sections of the livers of patients who had died with hepatic diseases and the livers of patients who had succumbed to other illnesses.

METHOD

Gomori's² method as modified by Kabat and Furth³ was employed. There were some minor alterations of the technic previously described, as follows. The time during which sections were incubated in the buffer-phosphatase mixture was prolonged to twelve to fourteen hours. If, however, abundant amounts of phosphatase were revealed, the sections were incubated for only two hours. No counterstain was used. Control sections were treated in an identical manner save for the fact that the glycerophosphate was omitted from the incubation mixture. In most of the cases, material was used only if the autopsy was done within eight hours or less after death. In a few instances a longer period had elapsed between death and autopsy. In control experiments, carried out on animal organs, it was seen that the time factor up to forty-eight hours was negligible.

ALKALINE PHOSPHATASE IN THE NORMAL LIVER

The liver of a 22 year old man who died a violent death and on whom the autopsy was done within half an hour was studied, as well as livers from 36 patients ranging in age from 6 months to 72 years. The livers of these patients, who had died from various diseases, were normal grossly and microscopically.

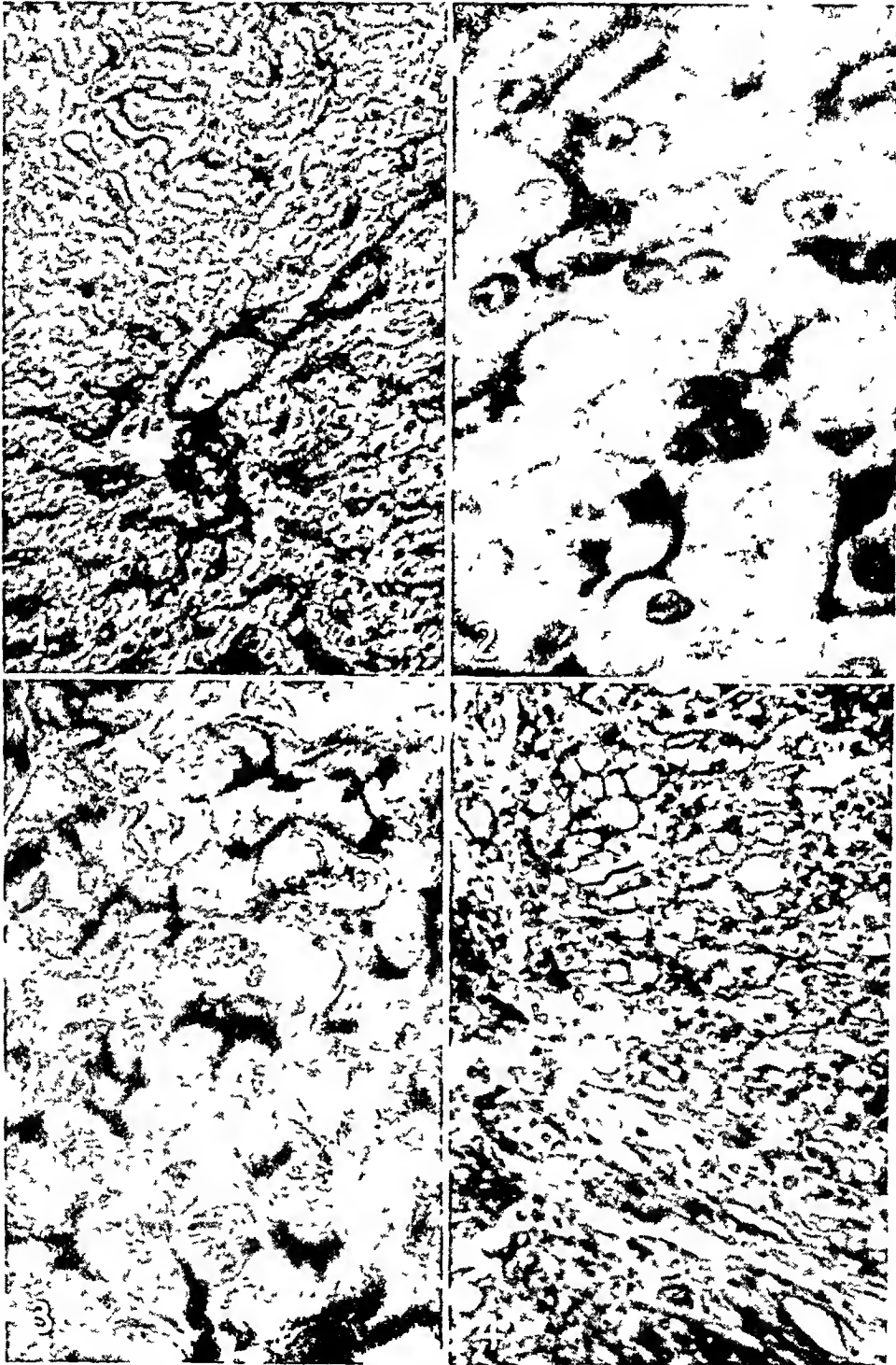
The cytoplasm stained only faintly, with occasional cell groups showing more intense reactions. In general the nuclei contained the enzyme, in the chromatin, the nucleoli and the nuclear membrane.

From the Laboratories of the Division of Pathology, Mount Sinai Hospital, New York, and the Elizabeth A. Horton Memorial Hospital, Middletown, N. Y.

1 (a) Wachstein, M. Arch Path 40: 57, 1945. (b) Wachstein, M., and Zak, F. G. Proc Soc Exper Biol & Med 62: 73, 1946.

2 Gomori, G. Proc Soc Exper Biol & Med 42: 23, 1939.

3 Kabat, E. A., and Furth, J. Am J Path 17: 303, 1941.



FIGURES 1 TO 4
(See legends on opposite page)

Occasionally, the entire nucleus took the stain. There was a varying degree of phosphatase activity in the walls of the sinusoids and in the Kupffer cells. In cases with much activity in the sinusoidal walls, there was often uneven staining. Phosphatase was demonstrated in a varying degree in the nuclei of the epithelium of the bile ducts. The endothelium of the larger veins and particularly of the small arteries and arterioles was frequently the site of conspicuous activity. The lymphocytes found in the portal fields showed inconstant amounts of phosphatase. The bile capillaries likewise displayed considerable variation in their staining reaction. In some instances, including the case of violent death, they were well outlined, while in others they were not visible (figs 1 and 2). They appeared as fine black lines, occasionally there was a distinct lumen. On cross section they were observed as minute black dots. In several livers, apparently normal, an incubation period longer than two hours caused loss of the sharp delineation of the bile capillaries and produced a widening of the stained zone.

Not infrequently fine black granules were scattered in the vicinity of the bile capillaries. It is probable that some of this activity is localized in the Golgi apparatus. Alkaline phosphatase activity was first demonstrated in the Golgi zone of the intestinal epithelium⁴ and has also been observed in the liver cells⁵. However, care must be taken in interpreting dark granules in liver cells as signifying phosphatase activity. Hemosiderin granules take a dark color owing to the formation of iron sulfide. Lipofuscin usually can be recognized by the more yellowish tinge, although occasionally it takes a darker color similar to that of ceroid, the pigment of experimental dietary cirrhosis^{1a}. In addition, calcium, which may sometimes appear in small granules, will also stain black. It is therefore necessary to check the specificity of the staining reaction by means of control sections incubated in a sub-

4 Emmel, V. M. *Anat. Rec.* **91** 39, 1945.

5 Deane, H. W., and Dempsey, E. W. *Anat. Rec.* **93** 401, 1945. Dempsey, E. W., and Wislocki, G. B. *Physiol. Rev.* **26** 1, 1946.

EXPLANATION OF FIGURES 1 TO 4

Fig 1—Alkaline phosphatase activity in the liver of a 22 year old man who died a sudden violent death. The sites of activity stain dark. There is prominent activity of the walls of the sinusoids. The bile capillaries appear as fine black lines. $\times 125$

Fig 2—A similar preparation of the same liver at a higher magnification. $\times 600$

Fig 3—Alkaline phosphatase activity in the liver of a patient with obstructive jaundice. The bile capillaries show conspicuous activity. $\times 450$

Fig 4—Alkaline phosphatase activity in the liver of a child with diffuse acute necrosis of liver cells. The cells in the periportal areas are preserved but show fatty changes, while all other cells are necrotic. The necrotic cells show a normal amount of enzymatic activity. $\times 125$

strate mixture without glycerophosphate in each single case in which black granules are visualized

OBSTRUCTIVE JAUNDICE

One biopsy and 9 autopsy specimens were studied. Obstruction of bile ducts occurred as a sequela of carcinoma of the gallbladder in 3 livers, of the pancreas in 2, of the hepatic ducts in 2, of the stomach in 2 and of the breast in 1. Metastases were found in 7 of these livers. As table 1 shows, a considerable increase of serum bilirubin and of alkaline phosphatase was present in all cases in which these determinations were carried out.

TABLE 1—*Obstructive Jaundice*

| Case | Diagnosis | Age, Yr | Blood Findings | Alkaline Phosphatase in Bile Capillaries |
|------|-------------------------------|---------|--|--|
| 1 | Carcinoma of gallbladder | 67 | Severe jaundice—no data available | Moderately increased, but not uniformly |
| 2 | Carcinoma of pancreas | 65 | Icteric index 80, alkaline phosphatase 19 Bodan sky units | Uniformly markedly increased |
| 3 | Carcinoma of gallbladder | 74 | Icteric index 57 | Markedly increased, but not uniformly |
| 4 | Carcinoma of gallbladder | 74 | Icteric index 95, alkaline phosphatase 14 Bodan sky units | Not increased |
| 5 | Carcinoma of breast | 54 | No data available | Uniformly increased |
| 6 | Carcinoma of common bile duct | 53 | Icteric index 60, alkaline phosphatase 31 King Armstrong units | Not increased |
| 7 | Carcinoma of hepatic duct | 65 | Icteric index 80, alkaline phosphatase 36 King Armstrong units | Moderately but not uniformly increased |
| 8 | Carcinoma of stomach | 52 | Severe jaundice—no data | Markedly uniformly increased |
| 9 | Carcinoma of stomach | 52 | Alkaline phosphatase 51 King Armstrong units | Markedly uniformly increased |
| 10 | Carcinoma of pancreas | 74 | Icteric index 21, alkaline phosphatase 16 King Armstrong units | Moderately increased, but not uniformly |

The cytoplasm of the liver cells showed little or no increase in alkaline phosphatase. The necrotic cells occasionally seen contained normal amounts of enzyme except in 2 specimens in which the reaction was stronger. In several livers the bile capillaries revealed considerable dilatation. In many fields they showed considerable widening of the stained zone (fig 3). Such changes in the bile capillaries were uniform in 4 livers while in 1 they were marked in some fields and not present in others. Only moderate and not uniform increase in phosphatase activity of bile capillaries was seen in 3 and no increase in the remaining 2 livers. In several obstructed livers the sinusoids presented a prominent staining reaction, a phenomenon observed in the livers of dogs with experimental obstruction of the common bile duct. Infiltrating lymphocytes showed a varying degree of activity. Bile pigment as well as bile thrombi appeared green in these sections. Occasionally

bile thrombi took a dark stain. This was not due to phosphatase activity, however, since in control sections the same color was seen.

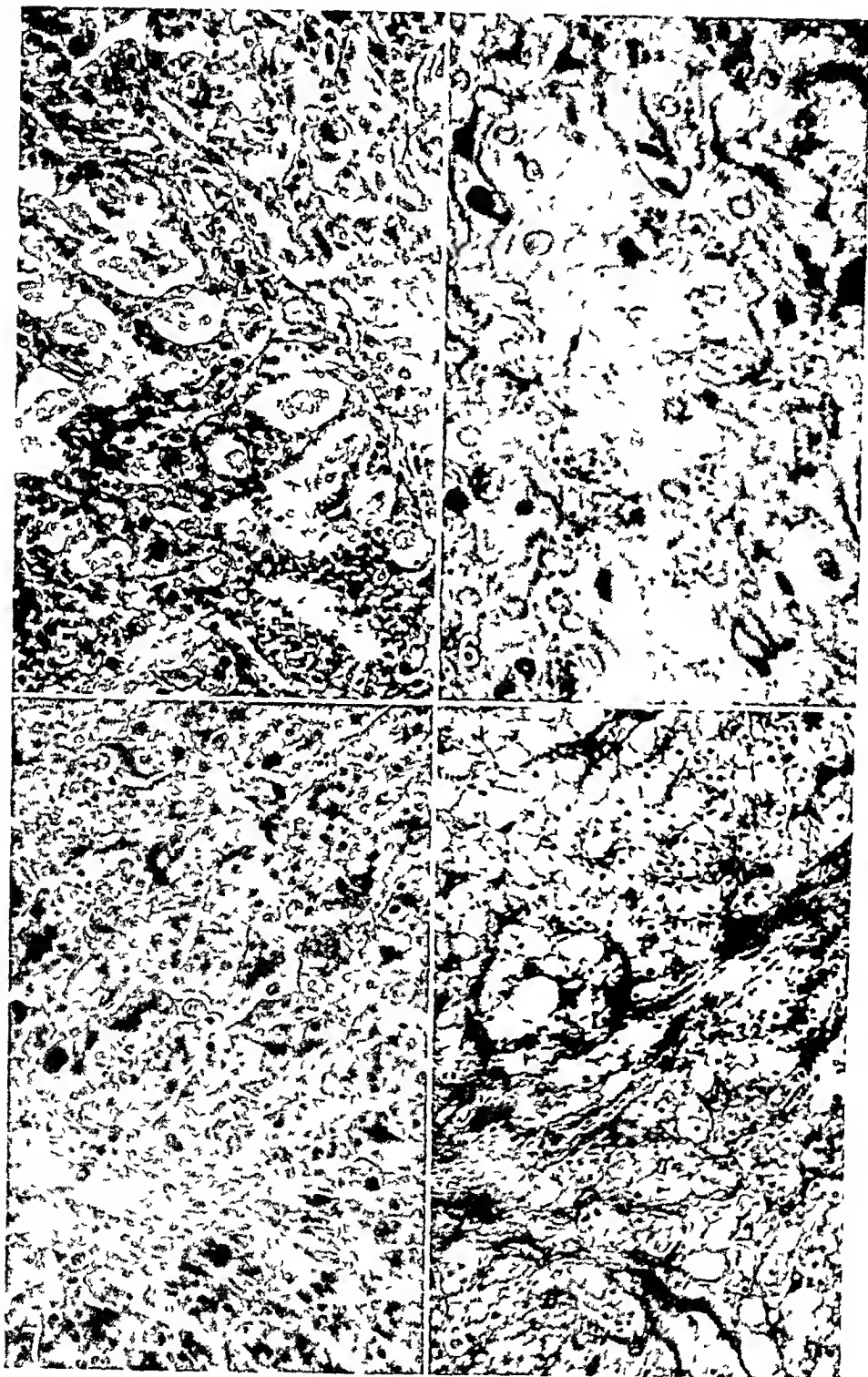
Some phosphatase activity was seen in the cells composing the metastatic tumor tissue. It was seen mostly in the chromatin and the nuclear membrane and only occasionally in the cytoplasm.

LIVER CELL NECROSIS

Three livers showing extensive acute necrosis of the hepatic cells and 4 with focal acute changes were studied. One liver with subacute yellow atrophy and another showing subacute hepatitis, from a patient with pernicious anemia, were included. In none of these livers was there an appreciable increase of alkaline phosphatase in the remaining undamaged liver cells. The necrotic cells did not show an increase in enzymatic activity (fig 4). Occasionally, these cells were somewhat more stained, but proper evaluation could not be made since they were frequently shrunken. Some contained a dark pigment. This at first gave the impression of increased phosphatase activity, but the granules stained in control sections did not signify this. Infiltrating leukocytes, lymphocytes and plasma cells showed a varying degree of activity. Bile capillaries between remaining liver cells were, for the most part, devoid of phosphatase activity. In necrotic areas, most of the walls of the sinusoids retained their activity. In the 2 livers with subacute inflammation, prominent activity was exhibited by proliferating fibroblasts and infiltrating lymphocytes (fig 5), in one specimen there was marked activity of the bile capillaries, while in the other they took the stain only faintly. A patient who died two days after extensive burns had clusters of hydropic cells in the liver. These showed much less phosphatase than the adjacent liver cells. However, this may have been due to the distention of cells and may therefore not have represented an actual decrease in phosphatase activity (fig 6). Cells of this kind occurring after experimental poisoning with chloroform and carbon tetrachloride show a similar behavior.¹¹

FATTY LIVER

Fatty changes were present in a number of specimens. In several only occasional fat droplets were seen, while in others, these changes were extensive. Phosphatase activity was missing in the location where the fat droplets had been present (figs 4 and 8). There was no accumulation of phosphatase in the remaining cytoplasm. The nuclei and walls of the sinusoids showed activity to the same extent as those in control sections. The appearance of liver cells containing fat was identical with that previously described in various experimental conditions.¹² Whether the disappearance of the enzyme seen in these preparations of fatty livers constitutes a real decrease can be determined



FIGURES 5 TO 8
(See legends on opposite page)

only with chemical methods. It is possible, although unlikely, that phosphatase somehow bound to the fat is removed together with the fat during the process of fixation and embedding.

CIRRHOSIS OF THE LIVER

Sections of 8 livers with Laennec cirrhosis, 2 with hypertrophic cholangiolitic cirrhosis, 2 with toxic cirrhosis and 1 with obstructive biliary cirrhosis were examined. In several of these organs there was also hepatocellular damage of recent origin. These patients had increases of bilirubin and phosphatase in their serum (table 2).

TABLE 2—*Cirrhosis of the Liver*

| Case | Diagnosis | Age, yr | Blood Findings |
|------|---|---------|---|
| 1 | Laennec cirrhosis and hepatitis | 49 | Jaundice—no data |
| 2 | Laennec cirrhosis and hepatitis | 69 | Icteric index 80 |
| 3 | Laennec cirrhosis and cancerous hepatoma | 60 | Alkaline phosphatase 33 King-Armstrong units |
| 4 | Laennec cirrhosis and hepatitis | 46 | Alkaline phosphatase 28 King Arm strong units, icteric index 39 |
| 5 | Laennec cirrhosis and hepatitis | 55 | Alkaline phosphatase 16 King Arm strong units, icteric index 60 |
| 6 | Laennec cirrhosis | 65 | No jaundice—no data available |
| 7 | Laennec cirrhosis | 71 | Alkaline phosphatase 13 King Arm strong units, icteric index 6 |
| 8 | Laennec cirrhosis | | |
| 9 | Hypertrophic cholangiolitic cirrhosis | 42 | Alkaline phosphatase 18 King Arm strong units, icteric index 6 |
| 10 | Hypertrophic cholangiolitic cirrhosis | 47 | Icteric index 16 |
| 11 | Toxic cirrhosis | 10 | Alkaline phosphatase 29 King Arm strong units, icteric index 27 |
| 12 | Toxic cirrhosis | 11 | Alkaline phosphatase 13 King Arm strong units, icteric index 90 |
| 13 | Obstructive biliary cirrhosis (14 years after traumatic laceration of common bile duct) | 69 | Alkaline phosphatase 49 King Armstrong units |

In most of the undamaged liver cells alkaline phosphatase activity was normal. There was no increase of the enzyme in necrotic cells. In some of the livers, there was a considerable increase of enzymatic

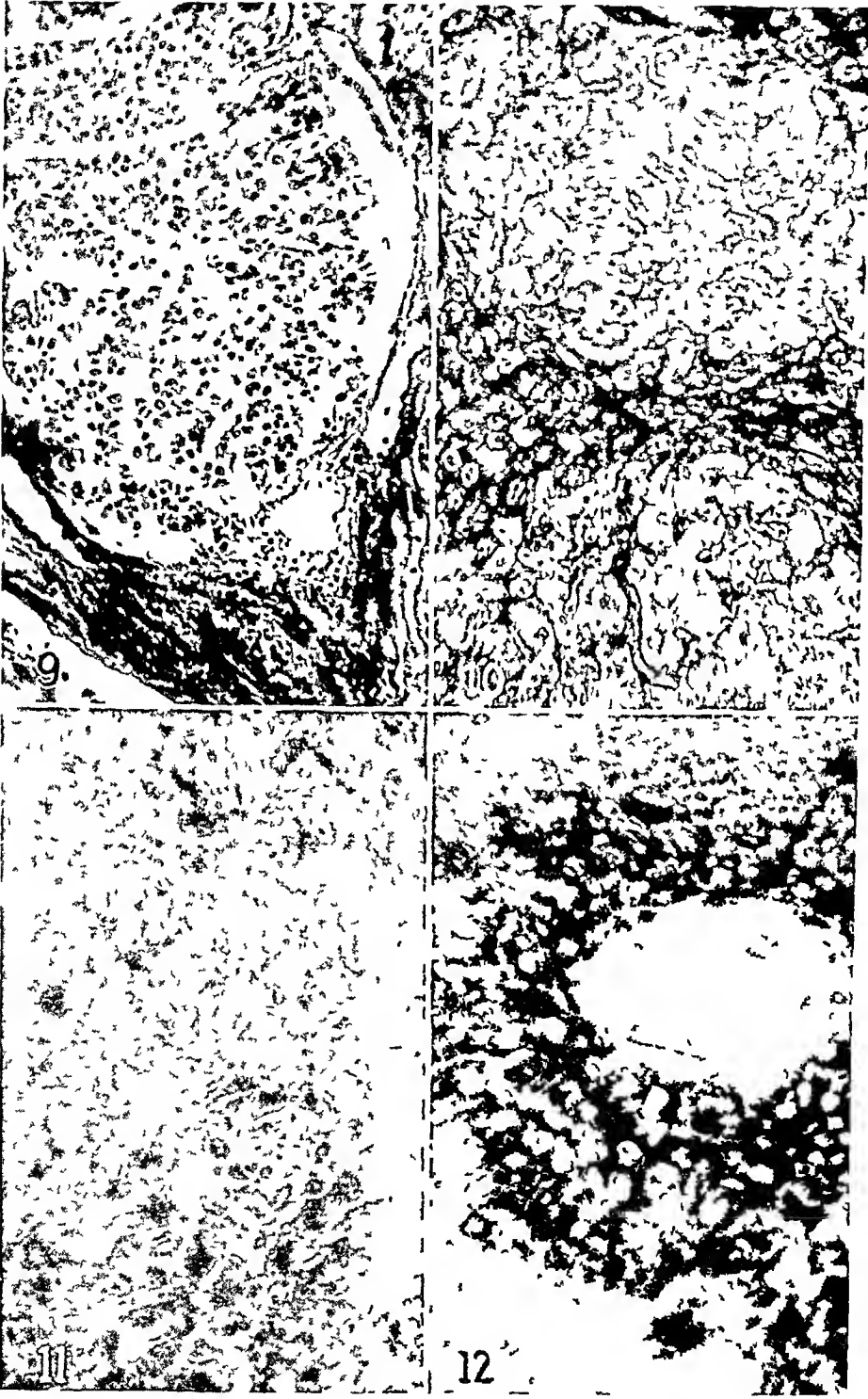
EXPLANATION OF FIGURES 5 TO 8

Fig 5—Alkaline phosphatase activity in the liver of a patient with subacute yellow atrophy of the liver. There is conspicuous activity in the proliferating connective tissue and the inflammatory cells. The remaining liver cells show a normal amount of phosphatase $\times 125$.

Fig 6—Alkaline phosphatase activity in the liver of a patient who died because of extensive burns. Hydropic liver cells show apparently a decrease in enzymatic activity $\times 180$.

Fig 7—Alkaline phosphatase in the liver of a patient with Laennec's cirrhosis and superimposed hepatocellular damage. There is considerable activity in some of the bile capillaries $\times 125$.

Fig 8—Alkaline phosphatase in the liver of a patient with Laennec's cirrhosis in a fatty stage. The proliferating connective tissue reveals phosphatase activity. The liver cells infiltrated by fat are devoid of enzymatic activity $\times 125$.



FIGURES 9 TO 12
(See legends on opposite page)

activity in the bile capillaries, and occasionally in the cytoplasm of cells surrounding those capillaries there was an increase (fig 7) Similar to the observations in obstructive jaundice, this increase was not uniform There was considerable variation in the activity of the connective tissue separating the pseudolobules The infiltrating lymphocytes contained a varying amount Phosphatase activity was decreased in fat-containing cells (fig 8)

There was 1 liver with cancerous hepatoma in association with Laennec's cirrhosis The amount of alkaline phosphatase activity in the tumor cells was similar to that in normal livers Phosphatase was localized mainly in the chromatin and the nuclear membrane, the cytoplasm contained little (fig 9) A normal distribution of phosphatase was also seen in 2 livers with benign adenoma

In 2 livers hypertrophic cholangiolitic cirrhosis, and in 1 liver obstructive biliary cirrhosis, was found The picture in each of these was not different from typical Laennec cirrhosis So-called "bile duct sprouts" in the connective tissue separating the pseudolobules did not show much activity The 2 livers with toxic cirrhosis showed conspicuous activity in the proliferating connective tissue and the infiltrating lymphocytes (fig 10), in one, bile capillaries showed occasional dilatation, while in the other some of the liver cells showed an increased amount of cytoplasmic phosphatase activity

LEUKEMIA

Livers from 4 patients with acute and 2 with chronic myeloid leukemia as well as from 1 with chronic lymphatic leukemia and 1 with lymphosarcomatosis were studied In 2 organs a diffuse moderate increase of cytoplasmic phosphatase activity was seen Otherwise, in distribution and amount of cytoplasmic activity the sections were similar to the control sections Infiltrating leukemic cells showed moderate phosphatase activity, especially in the nuclei

EXPLANATION OF FIGURES 9 TO 12

Fig 9—Alkaline phosphatase in the liver of a patient with hepatic carcinoma In distribution and amount of enzymatic activity the tumor cells are similar to normal liver cells The surrounding connective tissue is very active $\times 100$

Fig 10—Alkaline phosphatase in the liver of a patient with toxic cirrhosis There is conspicuous activity of the proliferating connective tissue, while the liver cells show a normal distribution $\times 125$

Fig 11—Alkaline phosphatase in the liver of a patient with moderate chronic passive congestion of the liver There is diffuse increase in cytoplasmic phosphatase $\times 125$

Fig 12—Alkaline phosphatase in the liver of a patient with severe passive congestion of the liver There is conspicuous activity in the bile capillaries of the undamaged cells in the periportal fields, while the centrally located damaged cells show a lessened amount of enzymatic activity $\times 100$

INCREASE IN CYTOPLASMIC PHOSPHATASE

An increase in cytoplasmic alkaline phosphatase was found in the livers of 12 patients who died of various diseases. Jaundice was absent in each instance. Three of the patients died of cardiac failure, 1 of lymphosarcomatosis, 1 of acute leukemia, 1 of carcinoma of the esophagus, 1 of carcinoma of the hepatic flexure, 2 of chronic nephritis, 1 of bleeding peptic ulcer, 1 of influenzal laryngotracheitis and 1 of diabetes mellitus and syphilitic aortitis. The increase in cytoplasmic phosphatase activity after fourteen hours of incubation was only moderate in some instances, quite striking in others (fig 11). In several livers many of the bile capillaries showed increased phosphatase.

There was an increase in the phosphatase activity of the cytoplasm in a number of livers with chronic congestion, in others not. Where there was an increase it may not have been real but apparent, the appearance may have been due to condensation of the cytoplasmic constituents secondary to pressure. In the livers showing chronic congestion the sinusoidal walls were not infrequently separated from the liver cells and stained as fine black lines.

COMMENT

Since Gomori² and Takamatsu⁶ demonstrated a histochemical method for the visualizing of alkaline phosphatase activity in tissue sections a number of papers have been published by investigators employing this method. The manner in which alkaline phosphatase is distributed in the normal organs of different species has been established.⁷ Interesting observations have been made concerning the appearance of phosphatase in the developing embryo⁸ the fetal membranes and the placenta.⁹

It has been established that under various experimental conditions changes in alkaline phosphatase activity occur, especially in the kidney¹⁰ and the liver.¹ However not many observations have been made on human material. The distribution of the enzyme in normal organs as demonstrated with the microtechnical method has been described by Takamatsu⁶ Gomori^{7a} and Kabat and Furth.³ That in the nervous

6 Takamatsu, H. *Tr Soc path jap* **29** 492, 1938

7 (a) Gomori, G. *J Cell & Comp Physiol* **17** 71, 1941 (b) Bourne, G. *Quart J Exper Physiol* **32** 1, 1943 (c) Kabat and Furth³

8 Moog, F. *Biol Bull* **86** 51, 1944

9 Wislocki, G. B., and Dempsey, E. W. *Am J Anat* **77** 1, 1946, **78** 1, 1946. Hard, W. L. *ibid* **78** 47, 1946

10 Hepler, O. E., Simmonds, J. P., and Gurley, H. *Proc Soc Exper Biol & Med* **44** 221, 1940. Breedis, C., Flory, C. M., and Furth, J. *Arch Path* **46** 402, 1943. Wilmer, H. A. *J Exper Med* **78** 225, 1943, *Arch Path* **37** 227, 1944. Wachstein, M. *ibid* **38** 297, 1944, *J Exper Med* **84** 25, 1946

system has been described by Landow, Kabat and Newman¹¹ This enzyme has also been studied in neoplasms¹² Marked decrease of the enzymatic activity of diseased kidneys was noticed by Gomori^{12b} Takamatsu⁶ stated that damaged liver cells contain increased amounts of phosphatase, without giving further details

The present report concerns an attempt made to study the distribution of alkaline phosphatase in the livers of postmortem material, It is obvious that organs of patients who have died of various diseases are not in the same state of preservation as are the organs of experimental animals Although even forty-eight hours after death no significant changes are seen in the amount and the distribution of enzymatic activity in normal animal organs, it is possible that during the agonal phase in man changes in enzymatic activity may take place This is particularly true for the liver, in which postmortem changes occur sometimes very rapidly We wish to emphasize these possible sources of error The number of postmortem examinations of livers is comparatively small and will have to be supplemented by more biopsies Such a study of fatty and cirrhotic livers of patients with pellagra has been announced by Gillman and Gillman¹³ However, even considering all the obvious difficulties, we see an encouraging feature in the fact that changes occurring in human livers under conditions of disease resemble those occurring under similar experimental conditions

The distribution in the normal human liver was found similar to that demonstrated by Gomori^{7a} It resembled the picture seen in the livers of various animals, although, naturally, in the organs of normal animals the results are more uniform This applies particularly to the activity in sinusoids and bile capillaries Phosphatase activity of the latter was more regularly seen in the rabbit and the dog It was also found distinct in some of the normal human livers Further investigation will have to establish whether the varying activity of the bile capillaries is due to varying functional stages¹⁴

Interpretations of the increased phosphatase activity in some of the livers offer difficulties so long as no correlated chemical studies are carried out While the staining methods for the demonstration of alkaline phosphatase permit its localization, quantitative changes are more difficult to interpret An impressive increase of the staining reaction occurring in the cytoplasm under otherwise identical conditions is sug-

11 Landow, H , Kabat, E A , and Newman, W Arch Neurol & Psychiat 48 518, 1942

12 (a) Gomori, G Am J Path 19 197, 1943, (b) J Mt Sinai Hosp 11 317, 1945 (c) Landow and others¹¹ (d) Kabat and Furth³

13 Gillman, J, and Gillman, T Arch Path 40 239, 1945

14 Forsgren, E Skandinav Arch f Physiol 55 144, 1929, J Morphol 47 519, 1929

gestive of a real increase of enzymatic activity. Such an increase has been observed in the livers of mice fed starvation and protein-deficient diets and to a lesser degree in rats ^{1a}. It is probable that this increase signifies an intensification of metabolic processes which requires a greater amount of phosphatase.

The findings in necrotic liver cells are in general in good agreement with the previously reported experimental findings. It was seen that in livers damaged by chloroform, carbon tetrachloride and phosphorus the necrotic liver cells did not show a significant increase in enzymatic activity ^{1a}. Only in occasional specimens among the human livers was phosphatase activity of necrotic liver cells increased. Mostly it was normal or even decreased.

In the livers with biliary obstruction accentuation of the bile capillaries was marked in some, moderate in others and completely missing in still others. Not in a single instance, however, was the increase in phosphatase activity in bile capillaries as conspicuous as that seen in the canine liver after ligation of the common bile duct ^{1b}. While in some livers enzymatic activity was obviously increased in the cytoplasm in the close vicinity of the bile capillaries, there occurred only little increase in the remaining cytoplasm. The fact that in several livers no increased activity of the bile capillaries could be found is of particular interest. Since such a behavior was also observed in one biopsy specimen, which was immediately fixed in acetone, postmortem changes can be excluded. It seems probable that the liver cells in these instances have lost their ability to take up phosphatase from the blood stream. Animal experiments dealing with this problem are in progress.

In livers showing hepatocellular damage a varying behavior of phosphatase activity in bile capillaries was observed. In several with extensive necrosis of liver cells no stainable capillaries could be made out, while in others, including some in which damage of liver cells was superimposed on preexisting cirrhosis, a varying amount of the bile capillaries showed prominent activity. Such widening of bile capillaries may be caused by intrahepatic obstruction due to hepatocellular dysfunction. However, a considerable increase in phosphatase activity in bile capillaries may occur without obvious cause, as illustrated in the following case. A 54 year old man died because of occlusion of the right coronary artery with subsequent myomalacia and perforation of the interventricular septum. The liver showed severe passive congestion. The cells in the centrally congested fields showed decreased enzymatic activity, while the remaining undamaged liver cells in the periphery showed considerable increase in enzymatic activity of the bile capillaries (fig. 12). The remaining cytoplasm showed normal amounts of phosphatase. Clinically and at autopsy jaundice was not present. In none of the other livers in which severe congestion occurred were similar changes seen.

Metastatic tumor tissue showed activity mostly in the nuclei. Kabat and Furth³ did not notice any activity in the tumor cells in cases of carcinoma of the breast, the stomach and the large intestines and hypernephroma. The moderate activity seen in our sections is obviously due to the fact that they were incubated longer in the substrate mixture. Phosphatase activity was also seen in the nuclei of a primary carcinoma of the liver and in those of two benign adenomas and to a lesser degree in the cytoplasm, resembling that observed in normal liver. Among animals, marked increase of phosphatase activity was found in the cancerous hepatoma of the rat, while the hepatoma of the mouse showed a decreased amount.¹⁵

The liver cells in various forms of cirrhosis showed preponderantly a normal activity of phosphatase. Occasional cells, which were located in the vicinity of bile capillaries showing conspicuous activity, revealed some increase in cytoplasmic activity. The considerable increase in phosphatase noticed in adenomatous areas in the experimental dietary cirrhosis of the rat^{1a} was not seen in human livers. A prominent feature in 2 livers showing toxic cirrhosis as well as in several with Laennec's cirrhosis was the considerable activity of the proliferating connective tissue. This was also seen in 2 livers with subacute diffuse hepatitis. The great activity of the young connective tissue of the rat's liver was first described by Fell and Danielli.¹⁶ In cases of cirrhosis the conspicuous amount of phosphatase presumably signifies a still active growth of connective tissue, while its absence is indicative of a quiescent stage.

In the livers of 7 patients who died of leukemia and of 1 who had lymphosarcomatosis, the infiltrating cells showed a varying degree of moderate phosphatase activity. Previously, absence of phosphatase activity had been found in the immature myeloid cells both of leukemic and normal persons, observed in bone marrow and peripheral blood, and a considerably lessened activity in the mature cells of patients with chronic myeloid leukemia.¹⁷ A different behavior was observed in the staining reaction of lymphocytes in blood films and tissue sections. While in blood films lymphocytes were consistently devoid of phosphatase, in lymph nodes and also in chronic inflammatory foci they showed activity of a varying degree. A similar observation had been previously made by Gomori. The reason for this difference is obscure. However, it is unlikely that the difference was due to the fact that the examinations were carried out on different preparations, since in cases of

¹⁵ White, J., Dalton, A. M., and Edwards, J. E. *J. Nat. Cancer Inst.* **2** 539, 1942. Woodard, H. Q. *Cancer Research* **3** 159, 1943. Edwards, J. E., Dalton, A. J., and Andervont, H. B. *J. Nat. Cancer Inst.* **2** 555, 1942. Kabat and Furth.³

¹⁶ Fell, H. B., and Danielli, J. F. *Brit. J. Exper. Path.* **24** 196, 1943.

¹⁷ Wachstein, M. *J. Lab. & Clin. Med.* **31** 1, 1946.

infection polymorphonuclear leukocytes show conspicuous activity both in tissue sections and in blood films

The source of the increased amount of serum alkaline phosphatase is still controversial¹⁸. One theory maintains that the damaged liver releases a phosphatase-activating substance which accounts for the rise in enzymatic activity. This has been disproved¹⁹. According to another theory,²⁰ the damaged liver cells may produce an increased amount of phosphatase. In conformity with the behavior of necrotic liver cells under experimental conditions, there is no significant increase of phosphatase in disintegrating liver cells of man. The amount of enzymatic activity is small both in normal and in necrotic liver cells.

Experimental evidence brought forward by several authors²¹ supports the third theory that a rise in alkaline phosphatase is due to the inability of the liver cells to excrete the enzyme. This view is shared by Gutman and co-workers on the basis of clinical evidence²². We believe that the pictures in human livers stained for alkaline phosphatase activity can best be explained on the basis of this theory. In patients with obstructive jaundice, considerable retention of the enzyme in the bile capillaries occurs, although it never reaches the extent seen in dogs with biliary obstruction. The fact that this activity was absent in several cases of obstructive jaundice, as well as the irregularity in which such retention was seen in hepatocellular damage, strongly suggests that the functional state of the liver cells is of great importance for the excretion of the enzyme. It is probable that the liver cells which are damaged too severely lose their ability to take up the enzyme from the blood and excrete it. Sharnoff and co-workers,²³ on comparing the hepatic tissue changes in human postmortem material and changes in serum alkaline phosphatase activity, concluded that the degree of injury of hepatic cells was the factor determining the rise of serum alkaline phosphatase. That the functional state of the hepatic cells is important for the excretion of alkaline phosphatase is further strongly supported by the findings of considerable increases in serum alkaline

18 Cantarow, A, and Trumper, M. *Clinical Biochemistry*, Philadelphia, W B Saunders Company, 1945, p 460. Moog, F. *Biol Rev* **21** 41, 1946. Wachstein¹⁴.

19 Delrov, G E, and King, E J. *Biochem J* **38** 50, 1944.

20 Bodansky, A. *Enzymologia* **3** 258, 1937, *Proc Soc Exper Biol & Med* **42** 800, 1939. Freeman, S, Chen, Y P, and Ivy A G. *J Biol Chem* **124** 79, 1938.

21 Armstrong, A R, and Banting, F G. *Canad M A J* **33** 243, 1935. Maddock, S, Schmidt, G, and Thannhauser, S J. *Federation Proc* **1** 181, 1942.

22 Gutman, A B, Olsen, J B, Gutman, E B, and Flood, C A. *J Clin Investigation* **19** 129, 1940.

23 Sharnoff, J G, Lisa, F R, and Riedel, P A. *Arch Path* **33** 460, 1942.

phosphatase in dogs fed choline-deficient diets. The increase in enzymatic activity in the blood serum is concomitant with the impairment of the liver function, as proved by various tests,²⁴ but is not accompanied by jaundice. The histologic picture of these livers is characterized by severe fatty changes.²⁵

On the basis of the behavior of alkaline phosphatase activity in the liver the increase in serum phosphatase occurring in hepatic diseases can best be explained in the following way. The parenchymal cells are unable to excrete the enzyme either because of external obstruction or because of inability to take up phosphatase from the blood or because of a combination of both factors.

However, it should be remembered that alkaline phosphatase can be excreted in additional ways. It can leave the body in the pancreatic juice,²⁶ through the intestines,²⁷ and, in some species, through the kidneys.²⁸ Severe disturbance of the calcium and phosphorus metabolism occurring in hepatic diseases can in turn influence the production of bone phosphatase.²⁹ These extrahepatic factors are probably of considerable importance. They may explain the peculiar behavior of serum alkaline phosphatase in certain conditions, such as congenital atresia of the bile ducts, in which there is a normal amount of this enzyme in the serum.

SUMMARY

Alkaline phosphatase activity was studied in sections of livers of human postmortem material. A fairly regular distribution was found in livers from patients who had died from various diseases not involving the liver itself. Considerable increase of enzymatic activity was seen in bile capillaries in the livers of several of the patients having obstructive jaundice. Increased activity was seen in the bile capillaries also in some of the livers with hepatocellular damage but not regularly. Necrotic liver cells showed no significant increase of enzymatic activity. Considerable activity was seen in the proliferating connective tissue in livers of patients with subacute hepatitis and patients with toxic cirrhosis and in the livers of some of the patients with Laennec's cirrhosis. Meta-

24 McKibbin, J. M., Thayer, S., and Stare, F. J. *J. Lab. & Clin. Med.* **29** 1109, 1944. McKibbin, J. M., Ferry, R. M., Jr., Thayer, S., Patterson, E. G., and Stare, F. J. *ibid.* **30** 422, 1945.

25 Dutra, F. R., and McKibbin, J. M. *J. Lab. & Clin. Med.* **30** 301, 1945.

26 Nothmann, M. *Bull. New England M. Center* **6** 76, 1944.

27 Kosman, A. J., Kaulbersz, J. W., and Freeman, S. *Am. J. Physiol.* **138** 237, 1943.

28 Cantarow, A., Stewart, H. L., and McCool, S. G. *Proc. Soc. Exper. Biol. & Med.* **35** 87, 1936. Flood, C. A., Gutman, E. B., and Gutman, A. B. *Am. J. Physiol.* **120** 696, 1937.

29 Austoni, B., and Coggi, G. *Presse med.* **42** 1594, 1934. Morris, N., and Peden, O. D. *Quart. J. Med.* **6** 211, 1937.

static as well as primary tumor tissue showed moderate phosphatase activity of the nuclei. Infiltrating leukemic cells showed some phosphatase activity of the nuclei. In some of the livers which were not involved primarily by diseases, considerable increases of cytoplasmic phosphatase activity occurred. The behavior of phosphatase revealed in these sections favors the assumption that the increase of serum alkaline phosphatase in cases of damage of the liver is due to retention of the enzyme in the blood. The inability of the liver to excrete it may be caused by external obstruction or by cellular dysfunction. The importance of extrahepatic factors, however, should not be underestimated.

GRANULAR CELL MYOBLASTOMA

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MUCH HAS been written in recent years about a group of tumors most frequently designated by the names "granular cell myoblastoma," "myoblastic myoma," "myoblastoma" and "rhabdomyoma." In 1924 Klemperer¹ reviewed the literature and reported 50 cases of "myoblastoma of striated muscle." Howe and Warren² in 1944 brought the review of the literature up to date and made a thorough analysis of 114 additional cases. Since then, new cases have been reported by Hartz,³ Ravich, Stout and Ravich⁴ and Crane and Tremblay.⁵

The distribution of the sites of these tumors is rather diffuse. Converting the figures of Crane and Tremblay⁵ into percentages, one finds that 37.6 per cent of the reported sites of myoblastoma were in the tongue, while 20.4 per cent were in the skin and the subcutaneous tissue. The others were in the following tissues in descending order of incidence: muscle, maxilla, breast, larynx, vocal cord, mandible, lip, trachea, bronchus, ear and alveolar process.

The various authors are fairly well agreed on the histologic characteristics of tumors of this group. Two types of cells are described. Cells of the first type are large, pale, granular and polyhedral or ovoid. Those of the second type are larger, elongated and finely granular and are arranged in ribbon-like syncytial masses. Cross and longitudinal striations have been observed in some of these cells. The cytoplasm tends to be acidophilic, but Horn and Stout⁶ reported great variation in the number, the size, the density and the depth of acidophilic staining of the characteristic granules. This may cause difficulty in diagnosis, especially when combined with a so-called organoid pattern of growth. In this pattern the cells have a pseudoalveolar arrangement frequently near or around thin-walled blood vessels, giving the growths a strong resemblance to tumors arising from endocrine glands.⁷ The nuclei are

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1 Klemperer, P. *Am J Cancer* **20** 324, 1934

2 Howe, C W, and Warren, S. *Surgery* **16** 319, 1944

3 Hartz, P H. *Am J Clin Path* **14** 582, 1944

4 Ravich, A, Stout, A P, and Ravich, R A. *Ann Surg* **121** 361, 1945

5 Crane, A R, and Tremblay, R G. *Am J Path* **21** 357, 1945

6 Horn, R C, and Stout, A P. *Surg, Gynec & Obst* **76** 315, 1943

7 Horn and Stout⁶ Hartz³

described as fairly regular, small, dense, with rare mitoses. The stroma is composed of fine strands of connective tissue, which supports either single cells or groups of cells. Some of these tumors are surrounded by a definite capsule, but the majority are nonencapsulated and infiltrating.

To date there is no agreement or any conclusive evidence in the literature concerning the exact origin of myoblastoma. Opinion seems to lean toward Klinge's⁸ dysontogenetic theory, which states that tumors of this group arise from retained or misplaced primitive myoblasts which failed to develop into adult muscle tissue. Abrikossoff,⁹ in reporting the first cases of myoblastoma, interpreted the tumors as arising from primitive muscle cells which developed in an attempt to repair a degenerative process following injury or inflammation, not from embryonal cell rests. Later he conceded that myoblastoma arising in an area devoid of striated muscle might originate from primitive myoblasts representing an embryologic rest. Gray and Gruenfeld¹⁰ maintained that convincing evidence is lacking of the uniformly myogenous nature of various intracutaneous, submucous, intramuscular and lingual tumors described as myoblastoma. They accepted the myogenous nature of the cells in lingual tumors but expressed the belief that these lesions are the result of a degenerative process in skeletal muscle. However, they could not explain the proliferation or the invasiveness of the tumor cells. Some observers noted the xanthomatous appearance of myoblastoma, but special stains failed to reveal the presence of fat in the cells. Due to the granularity of the cytoplasm the tumors have been described as "storage cell tumors" arising from muscle cells or undifferentiated mesenchyme.¹¹ The possibility that the large granular cells may be histiocytes prompted Tuta and Schmidt¹¹ to use various staining methods to determine the nature of the cytoplasmic granules. They thought that glycogen and a mucin-like substance may have been present in the cytoplasm of the cells in question. Their results were not conclusive, since the tissue had not been properly fixed in absolute alcohol.

Granular cell myoblastoma is considered to be a benign tumor. However, Howe and Warlen² reported a total of 14 cases in which it showed cancerous characteristics, but in only 3 of these were metastases demonstrated. This was followed by a report from Ravich, Stout and Ravich⁴ of a cancer of the bladder which was diagnosed as granular cell myoblastoma and which recurred after excision. At autopsy, extensive metastases were found, notably in the liver, the spleen, the lung, the

8 Klinge, F. *Verhandl d deutsch path Gesellsch* **23** 376, 1928.

9 Abrikossoff, A. *Virchows Arch f path Anat* **260** 215, 1926.

10 Gray, S. H., and Gruenfeld, G. E. *Am J Cancer* **30** 699, 1937.

11 Tuta, J. A., and Schmidt, F. R. *Arch Dermat & Syph* **46** 225, 1942.

skull and the pelvic tissues. Local recurrences following incomplete removal have been reported by Horn and Stout⁶ and Cappell and Montgomery¹². According to Howe and Warlen² when a myoblastoma shows (1) atypism of cells, (2) an excessive number of mitotic figures, (3) a spindle cell or sarcomatous pattern and (4) local invasion, it should be treated surgically as a cancer, especially if the patient is over 50 years of age.

REPORT OF CASES

CASE 1—During the course of a routine physical check-up, Mrs. F. J., a 49 year old white woman, was found to have a small painless nodule in the skin over the right scapula. This tumor was excised on May 2, 1944. The scar was inspected on June 19, 1946, and there was no evidence of recurrence.

The gross specimen consisted of an elliptic piece of skin, measuring 4 by 2.5 cm., with underlying subcutaneous tissue attached. In the center of this mass of tissue was a nodule measuring 1.7 cm. in diameter. The skin overlying the nodule was coarse and slightly roughened. On section the nodule was rather firm in consistency, yellowish gray and poorly circumscribed. Microscopic sections showed it to be composed of cells typical of granular cell myoblastoma. The squamous epithelium was slightly thickened over the mass. The tumor cells extended up to the basal layer of the epithelium but did not invade it. The entire corium except for a few hair follicles, sweat glands and bundles of collagen fibers was replaced by a fairly compact tumor, which possessed a fine connective tissue stroma. The tumor cells were elongated and polygonal, with an abundance of cytoplasm, which was pink staining and diffusely granular. The nuclei were small, round, pale staining and centrally located, without evidence of mitotic figures. Tumor cells were present deep in the subcutaneous fat. Occasionally, the cells had a vacuolated, foamy appearance suggestive of xanthoma cells, but special stains failed to reveal the presence of fat.

CASE 2—E. N., a 60 year old Negro woman, was seen on March 22, 1944, because of a nonpainful tumor of the right breast. Her only complaint was of itching of the skin over this tumor. Examination of the breast revealed a mass in the upper and outer quadrant. The tumor was not attached to the underlying tissue but was definitely attached to the skin, which was discolored in this area. There were no palpable axillary nodes. Simple mastectomy was done on March 23. The patient was last seen on Aug. 16, 1945, and there was no evidence of recurrence.

Only a portion of the breast was submitted for pathologic examination. It measured 8 by 5 by 4 cm. and was composed mostly of fatty tissue covered by pigmented skin. Included was a tumor mass, measuring 2.5 cm. in diameter. It was gray, firm, fairly well demarcated but not encapsulated. It was adherent to the skin. Microscopic sections showed the tumor to be composed of oval or polygonal cells, which were large and pink staining and contained an abundance of finely granular cytoplasm. The nuclei were small, deep staining and regular. The cellular outlines were indistinct in areas, and in other areas there was a pseudoalveolar pattern. Occasional ribbon-like syncytial arrangements of cells were also seen. Tumor cells extended up to the thin, normal-appearing squamous epithelium of the skin. Scattered throughout the mass were small collections of

¹² Cappell, D. F., and Montgomery, G. L. *J. Path. & Bact.* **44**: 517, 1937.

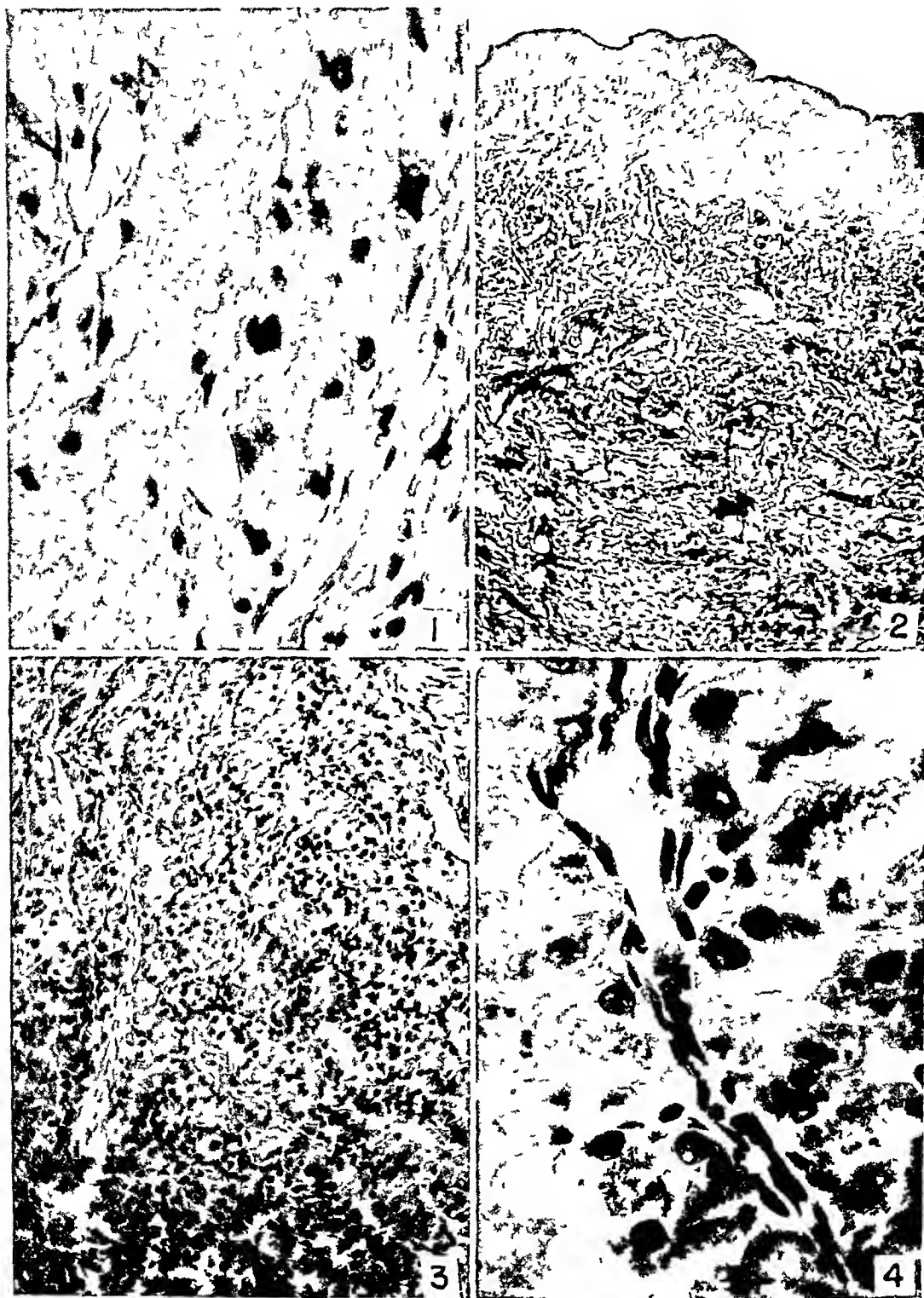


Fig 1 (case 2) —Cells of the tumor of the breast showing granularity of the cytoplasm and syncytial arrangement $\times 550$

Fig 2 (case 1) —Low power view showing the characteristic hyperplasia of the epidermis $\times 122$

Fig 3 (case 3) —An area in the tumor mass of the tongue showing the alveolar arrangement of the cells $\times 122$

Fig 4 (case 3) —Same as figure 3 $\times 550$

lymphocytes and occasional ducts. No definite stainable fat could be demonstrated in the tumor cells.

CASE 3—J. L. C., a 14 year old Negro youth, was first seen on Aug. 22, 1944, complaining of a painless growth located between the teeth and the tongue on the left side of his mouth, of four months' duration. Three months previously he noted that it interfered with his speech. Two years before the appearance of the growth he noticed a swelling, which was painless, below the left side of his jaw. Physical examination showed a mass arising between the tongue and the teeth on the left side, which extended to the right side of the floor of the mouth and displaced the tongue to the right. There was also a hard swelling under the left mandible, extending down into the neck. Biopsy was done on August 22 and on September 1. He was again seen on October 3, at which time excision of the tumor was considered but was not done. On his last admission, November 18, he complained of bleeding from under the tongue of four weeks' duration. On the day of admission the bleeding became profuse. Examination showed the site of the previous biopsy under the tongue to be granular, bleeding and apparently necrotic. Sterile cotton was packed under the tongue to control the bleeding, but this measure proved inadequate. In spite of blood transfusion and intravenous injection of fluids, his condition grew worse, and bleeding continued. Finally, November 24, the left external carotid artery was ligated, with the patient under pentothal sodium anesthesia. Following the operation, the patient had two attacks of respiratory failure, which were treated by artificial respiration and administration of respiratory stimulants. The respirations then became fairly regular, but the patient never regained consciousness. He died nine hours after the operation.

At autopsy the tongue, with tumor attached, was removed. The omohyoid muscle was almost completely destroyed by the growth, which seemed to arise from the under surface of the tongue near the base. The tumor measured 8 by 6 by 4 cm., was firm and rubbery in consistency and involved the entire under surface of the tongue except for a 2 cm. portion near the tip. There was an extensive erosion of the under surface of the tongue and the tumor proper. The walls of the resulting cavity were soft, friable, ragged and necrotic. The tumor involved the alveolar margin of the gum and destroyed a portion of the mandible on the left side. Microscopic sections showed typical large ovoid or polygonal pink-staining cells with an abundance of coarsely granular cytoplasm and regular small nuclei. The nucleoli were enlarged and unusually prominent. The majority of the cells were arranged in an organoid pattern of groups of from four to six cells. These groups were surrounded by thin connective tissue containing small capillaries. There were suggestive attempts at formation of lumens. Occasional syncytial masses of tumor cells with indistinct cellular outlines were seen. There was invasion and destruction of the striated muscle of the tongue. In other areas the tumor was necrotic and heavily infiltrated by neutrophils and lymphocytes. At the margin of these necrotic areas were numerous large, thin-walled dilated vessels, which were engorged with blood. The pathologic diagnosis of the tumor was myoblastoma.

CASE 4—G. H., a 26 year old Negro woman was first seen, Jan. 3, 1944, complaining of almost constant pain in the lower abdominal and pelvic regions of several years' duration. This pain had been increasing in severity, especially during her menstrual periods. The menses had been regular. She also complained of numerous nodules over her body, most of which had been present as long as she could remember. These nodules began as small pimples, causing no pain or discomfort other than slight itching. The skin over these masses had never

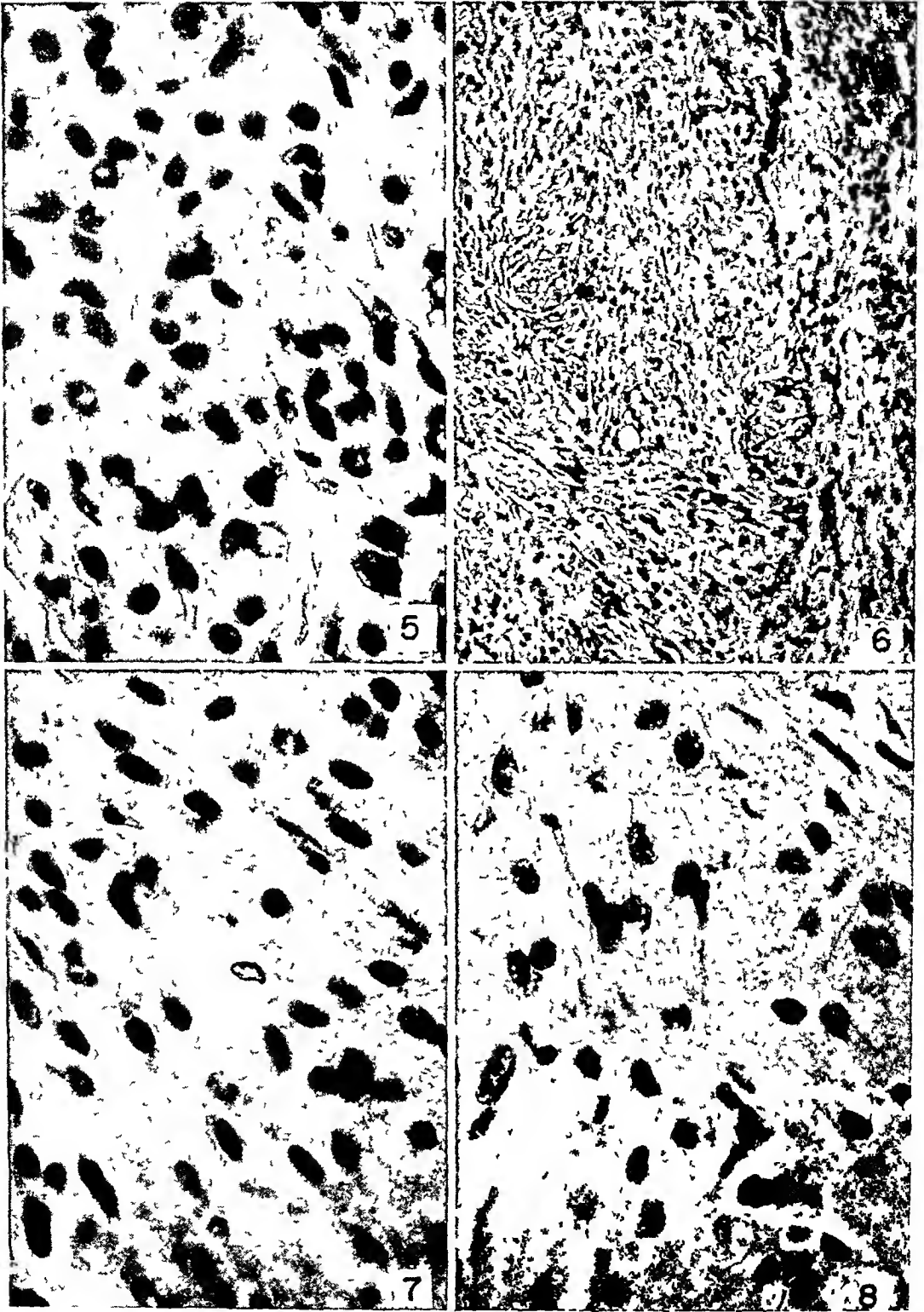


Figure 2

(See legend on opposite page)

become ulcerated or infected. Physical examination showed numerous firm subcutaneous nodules, which were freely movable except for their attachment to the skin. These masses were present in the scalp, an upper eyelid, the left side of the lower lip, the right axilla, the right lateral body wall above the pelvic brim, the left thigh and the vulva, between the shoulder blades, in the middle right toe and on the buttocks. They varied from a few millimeters to 4 cm in diameter. On pelvic examination the positive findings consisted of a vaginal stricture, a cervical polyp and an enlarged, nodular uterus. The adnexa could not be made out. On January 4, tumor masses were removed from the left thigh, the vulva and the right axilla. Panhysterectomy and bilateral salpingo-oophrectomy were done January 13 with removal of a retroperitoneal tumor. The uterus was enlarged and contained several subserous growths, diagnosed as leiomyoma, the largest of which measured 4 cm in diameter. The right ovary measured 6 by 6 by 5 cm. Except for a thin cortex, the organ had been completely replaced by a solid tumor mass, which had a soft, doughy feel. This mass was not encapsulated. The left ovary measured 5.5 by 4 cm by 3 cm. It contained two tumor masses similar in gross appearance to that in the right ovary. The larger mass measured 4 by 3 cm. The smaller was 1 cm in diameter. The retroperitoneal mass measured 7 by 6 by 3 cm. It had an irregular outer surface and was not encapsulated. On cut section its gross appearance was similar to that of the ovarian growth. In March 1945 a subcutaneous nodule was removed from the perineum. In the meantime several nodules had appeared over her body, none of which caused her any discomfort. She was seen on Oct 20 and Nov 30, 1945. On both of these occasions examination demonstrated that no pelvic masses were present.

On histologic study one was struck by the remarkable similarity of the tumor cells in the subcutaneous nodules, the ovarian tumors and the retroperitoneal mass. These were either spindle shaped, ovoid or polyhedral and fine to coarsely granular. The cutaneous and ovarian lesions showed a somewhat foamy appearance of the cytoplasm, which was almost entirely absent in the retroperitoneal tumor, in which many of the cells were plump and spindle shaped. The nuclei were fairly regular, with slight variations in staining characteristics but no evident mitoses. The early and late lesions of the skin were diagnosed as myoblastoma, as was the tumor found in the ovaries and the retroperitoneal mass.

COMMENT

All 4 cases were typical histologically of so-called granular cell myoblastoma. In the first 3 cases the tumor was nonencapsulated and locally invasive. In case 3 there was actual destruction of the neighboring tissue by the lingual neoplasm. In none of these cases were longitudinal or cross striations present, nor was there any definite pattern

EXPLANATION OF FIGURE

Fig 5 (case 4)—High power view of the tumor cells in the early lesion of the skin. $\times 550$

Fig 6 (case 4)—A typical field from the tumor in the right ovary, which was identical in histologic appearance with the tumor in the left ovary. $\times 122$

Fig 7 (case 4)—Section from the retroperitoneal mass showing a tendency of the cells to be more spindle shaped. The cytoplasm is finely granular. $\times 550$

Fig 8 (case 4)—Microscopic appearance of the tumor which was removed from the perineum a year after the pelvic operation. $\times 550$

to the arrangement of the cytoplasmic granules. Sections of the myoblastoma of the tongue failed to show any forms transitional from myoblastoma cells to striated muscle. However, there was a remarkable organoid or pseudoalveolar pattern to the arrangement of the myoblastoma cells which gave this tumor a strong resemblance to neoplasms arising from endocrine glands.

A new factor entered into the fourth case which was not present in the others, namely, the presence of metastatic nodules. If one can judge the age of a tumor by size, the primary growth should have been either the ovarian or the retroperitoneal. However, from the standpoint of the known age of a lesion, the fact that the axillary subcutaneous nodule had been present for ten years or more would suggest that this was the original lesion. Although it is unusual for tumors of the skin to metastasize in such a manner, the behavior of slow-growing malignant melanoma of the skin offers a counterpart to this particular picture.

SUMMARY

The third of the 4 cases of granular cell myoblastoma reported is unusual because the tumor caused the death of the patient through local invasion and erosion of a large vessel.

The fourth case is unusual because of the widespread subcutaneous tumors and the involvement of both ovaries. It is believed to be the first case of granular cell myoblastoma reported in which the ovaries were involved.

Cases 3 and 4 are included in this paper with the permission of Dr. Violet Keiller, pathologist of Hermann Hospital, Houston, Texas.

TOXICOLOGY OF 1,2-DICHLOROPROPANE (PROPYLENE DICHLORIDE)

III Pathologic Changes Produced by a Short Series of Daily Exposures

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AND

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THE COMPOUND 1,2-dichloropropane, $\text{CH}_2\text{ClCHClCH}_3$, is a colorless liquid with a characteristic and rather unpleasant odor. It has a specific gravity of 1.1593 at 20/20 C and a boiling point of 96.8 C. It is finding extensive use as a solvent and as an insecticide. A mixture of dichloropropane and dichloropropylene in various proportions is applied to the soil for killing nematodes on the roots of pineapple plants and a wide variety of truck crops. Dermatitis has occurred among workers using this mixture as a soil fumigant.

In 1932 Wright and Schaffer¹ described the pathologic changes occurring in dogs after oral administration of dichloropropane to test its anthelmintic properties. However, as far as could be determined, no pathologic or toxicologic studies of the effects of inhalation of this compound are recorded in the literature other than the first two papers of this series². Because our previous experiments were devised to permit study of the effects of a long-continued series of daily inhalation exposures, only a small number of animals that died or were killed after a few exposures to dichloropropane were studied histologically. In general, it was found that such animals showed far greater pathologic changes than those killed after surviving many exposures.

Accordingly, the following experiments were devised to permit more adequate study of the character, the development and the frequency of any early lesions caused by inhalation of 1,2-dichloropropane. Experiment A was devised to permit study of the pathologic changes occurring during a short series of daily exposures, and experiment B, to permit study of the changes following a single seven-hour exposure, to 2,200 parts per million (ppm) of dichloropropane for comparison.

From the Pathology Laboratory and the Industrial Hygiene Research Laboratory, National Institute of Health, United States Public Health Service

1 Wright, J. H., and Schaffer, J. M. *Am J Hyg* **16** 325, 1932

2 (a) Heppel, L. A., Neal, P. A., Highman, B., and Porterfield, V. T. *J Indust. Hyg & Toxicol* **28** 1, 1946. (b) Heppel, L. A., Highman, B., and Porterfield, V. T. *J Pharmacol & Exper Therap* **87** 11, 1946

In experiment A 30 young adult guinea pigs from our stock colony and 36 adult rats of the Sprague Dawley strain were exposed in the manner described in the preceding papers² to 2,200 ppm of 1,2-dichloropropane. Six additional guinea pigs and 6 rats of the same strain and approximately the same age and weight as the exposed animals were used as controls. These were kept in cages adjoining the experimental animals, were fed the same stock diet, previously described,^{2a} and were handled similarly except that they were not placed in the exposure chamber. Three guinea pigs were killed sixteen hours after the end of a seven hour exposure to 2,200 ppm of dichloropropane. The others were killed immediately after the last exposure as follows:

- 3 guinea pigs and 3 rats after one four hour exposure
- 3 guinea pigs and 3 rats after one seven hour exposure
- 5 guinea pigs and 6 rats after two seven hour exposures
- 5 guinea pigs and 5 rats after three seven hour exposures
- 4 guinea pigs and 5 rats after four seven hour exposures
- 5 rats after five seven hour exposures

One guinea pig died during and 3 several hours after the second exposure, and 2 died during and 1 after the third exposure. Six rats died several hours after one seven hour exposure, and 2 died during and 1 after the second exposure. The animals that died were not studied histologically.

In experiment B (single exposure) 33 young guinea pigs from our stock colony, each weighing 600 to 800 Gm, and 33 adult rats of the Sprague Dawley strain, each weighing 150 to 200 Gm, were given one seven hour inhalation exposure to 2,200 ppm of 1,2-dichloropropane. In addition, 3 unexposed guinea pigs and 3 rats served as controls. The exposed animals were killed at intervals after the single exposure as tabulated:

| | Animals Killed After Given Number of Days | | | | | | | | | | Animals That Died | Total | Controls |
|-------------|---|---|---|---|---|---|----|----|----|----|-------------------------|-------|----------|
| | 0 | 1 | 2 | 4 | 7 | 9 | 11 | 14 | 16 | 21 | | | |
| Guinea pigs | 3 | 5 | 5 | 3 | 5 | 3 | 2 | 2 | 2 | 3 | | 33 | 3 |
| Rats | 3 | 5 | 5 | 3 | 5 | 5 | | 5 | | | 2 | 33 | 3 |

Two rats that died shortly after the exposure were not studied histologically because of advanced autolysis.

Autopsies were performed on all the experimental animals immediately after they were killed. Many animals showed gross evidence of slight visceral congestion and a fatty liver, and cut sections of the adrenal glands of the guinea pigs often showed a dark hemorrhagic central portion. Tissues were fixed immediately in Orth's solution, and routine sections of the organs were stained with azure eosinate^{2a} and with iron hematoxylin and Van Gieson's solution of trinitrophenol, U S P (picric acid) and acid fuchsin. Paraffin sections of liver and spleen were examined for hemosiderin.³ Frozen sections of heart, liver and kidney were stained for fat by the method of Lillie and Ashburn.⁴

MICROSCOPIC CHANGES IN GUINEA PIGS

Certain lesions were seen in some of the guinea pigs used as controls, but such lesions were generally more extensive and severe in the exposed animals. These lesions included interstitial and lobular pneumonia, splenic suppuration and necrosis, and occasional small hepatic foci of coagulation necrosis, often margined by monocytes.

2a Lillie, R D. J Tech Methods **24** 43, 1944

3 Highman, B. Arch Path **33** 937, 1942

4 Lillie, R D, and Ashburn, L L. Arch Path **36** 432, 1943

The specific lesions, found only in exposed guinea pigs, were fatty degeneration of the liver and the kidneys and necrosis of the adrenal glands. In general, the fat in the liver was irregularly distributed as fine to moderately coarse intracellular globules throughout the parenchyma. The fat in the kidney occurred as fine globules confined chiefly to the basal portion of the epithelium of the convoluted tubules and thick portions of the loops of Henle. The amount of fat seen in microscopic sections of the liver and the kidney in experiment A (repeated exposures) was small to moderate in animals killed after one and two seven hour exposures to 2,200 ppm of 1,2-dichloropropane, large after three daily exposures and minimal in those killed after four exposures. In experiment B 1 of the 3 animals killed immediately after the single exposure showed a moderate and 1 a small amount of fat in the heart, the liver and the kidney. No significant amount of visceral fat was seen in any of the other animals in this experiment. Sections of liver of a limited number of animals in experiment B were prepared and stained for glycogen. Most animals had a marked decrease of glycogen in the liver immediately and twenty-four hours after the single exposure, a normal amount two days and a very large amount four days or more after the exposure. In paraffin sections of these glycogen-laden livers, most of the liver cells, particularly the centrilobular ones, appeared swollen, with pale, vacuolated cytoplasm.

Lesions in the adrenal cortex occurred in all exposed guinea pigs and varied in severity with the individual animal, the time elapsed since exposure and the number of exposures.

In guinea pigs killed immediately after single four hour and seven hour exposures, the inner portion of the adrenal cortex presented slight to moderate cytoplasmic oxyphilia and nuclear pyknosis, slight to marked congestion and structural disorganization and frequently one or more small areas of hemorrhage grading into hemorrhagic necrosis. In experiment A 1 animal killed after a single seven hour exposure showed diffuse hemorrhagic necrosis of the zona reticularis grading peripherally into a broad zone of congestion and hyaline and karyorrhectic necrosis.

The adrenal glands of guinea pigs killed sixteen, twenty-four and forty-eight hours after the end of a single exposure and immediately after two daily exposures presented a usually fairly well delineated area of cortical coagulation necrosis which involved all but a narrow outer rim of the cortex. In this necrotic area there was some distortion of structure with a variable amount of intercellular fibrinoid exudate. The cytoplasm of the necrotic parenchymal cells was deeply eosinophilic and sometimes vacuolated, and their nuclei were poorly stained and less often pyknotic, fragmented or apparently absent. The endothelial cells showed similar changes. Along the congested border of the necrotic area were occasional mononuclear cells and many neutrophils and fragmented nuclei, often forming a continuous or interrupted thin layer. Between this and the viable cortex and medulla was a narrow congested zone, which often showed some scattered neutrophils and, particularly around the medulla, sometimes graded into hemorrhagic necrosis.

Guinea pigs killed after three and four exposures showed similar cortical changes, but usually the viable cortex was slightly thicker and the layers of neutrophils near the congested borders of the necrotic area were broader and further removed from the viable cortex and medulla. The central portion of the necrotic area showed extensive karyolysis and often included one or more foci margined or infiltrated by neutrophils. The necrotic parenchymal cells in such foci were often hyalinized, poorly outlined or agglutinated.

In guinea pigs killed four and seven days after a single exposure the viable cortex was thicker than in more recently exposed animals. In the necrotic area

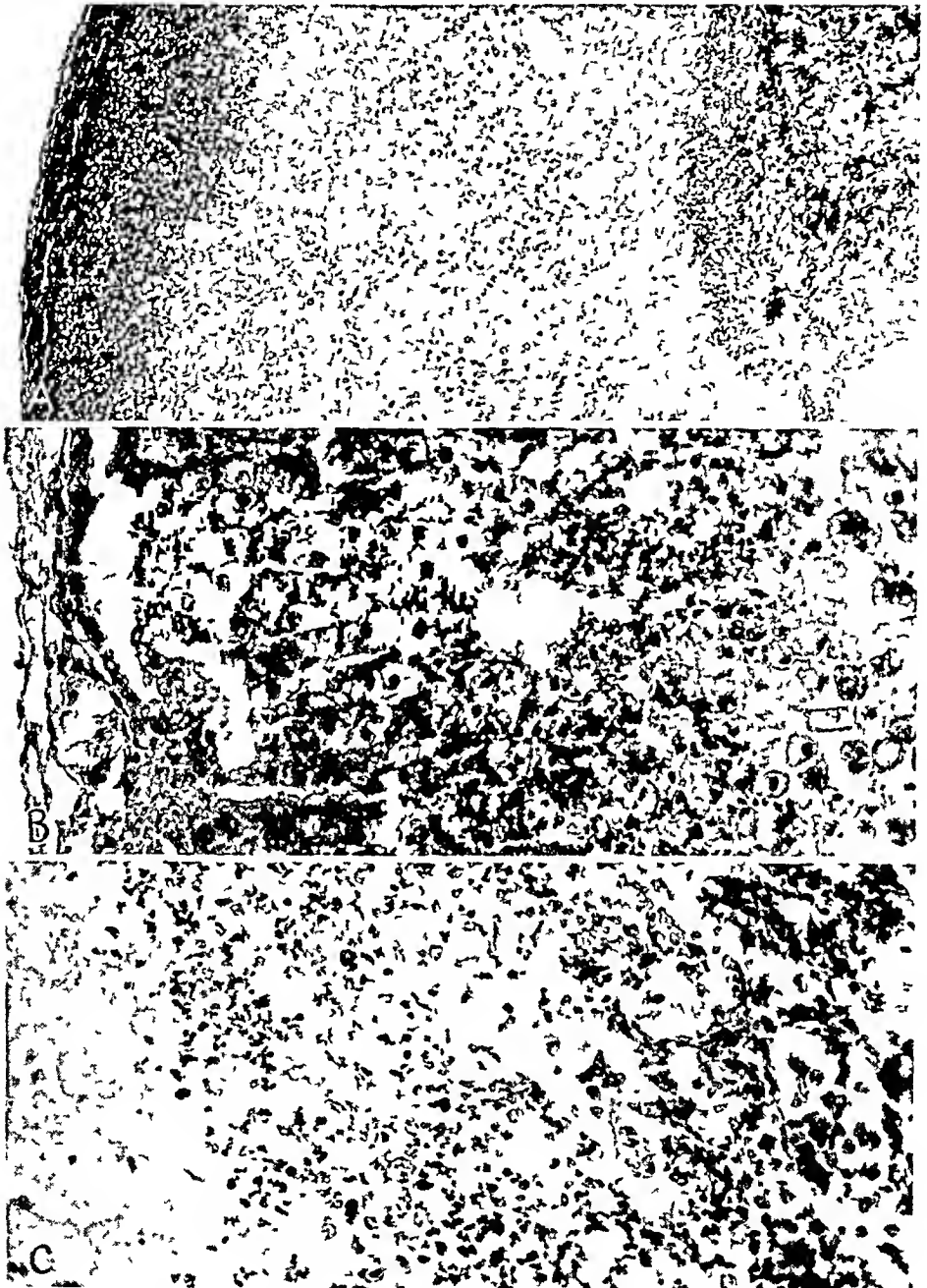


Fig 1—Photomicrographs of adrenal gland of a guinea pig killed immediately after two seven hour exposures to 2,200 ppm of 1,2-dichloropropane Azure eosinate stain

A, large cortical area of necrosis margined by leukocytes Congested medulla is on the right × 55

B, viable cortex and congested leukocytic zone marginating the necrotic area on the right × 250

C, congested medulla and leukocytic zone marginating the necrotic area on the left × 250

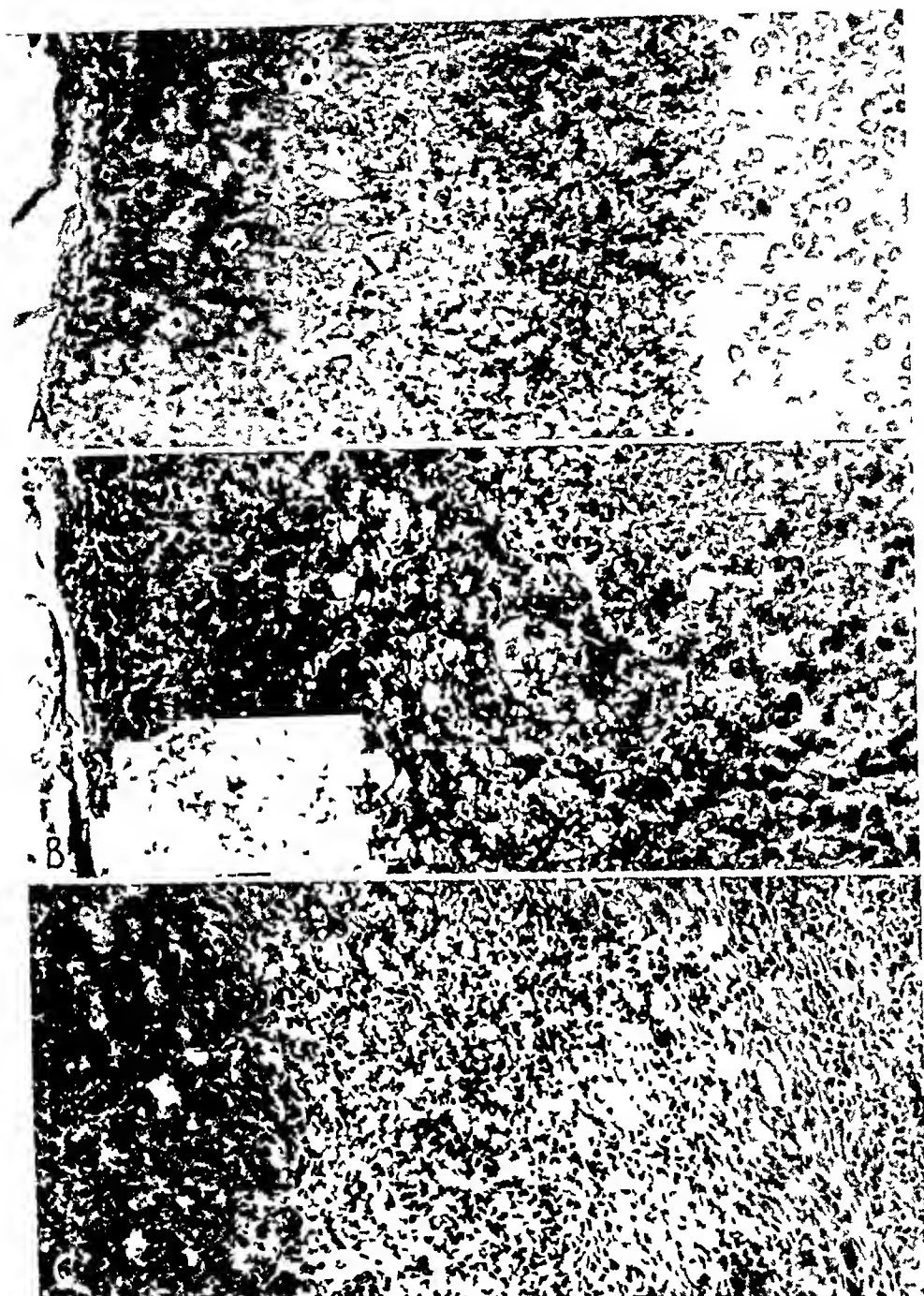


Fig 2—A, outer portion of adrenal cortex of a guinea pig killed twenty-four hours after a single seven hour exposure to 2,200 ppm of 1,2-dichloropropane. Note mitotic figures in the viable cortex Azure eosinate, $\times 150$

B, adrenal cortex of a guinea pig killed seven days after a single seven hour exposure to 1,2-dichloropropane Note the layer of epithelioid cells bordering the necrotic area Azure eosinate, $\times 150$

C, adrenal gland of a guinea pig killed eleven days after a single seven hour exposure to 1,2-dichloropropane Note the layer of epithelioid cells contiguous with the connective tissue layer marginating the medulla on the right Azure eosinate, $\times 150$

the central portion showed some scattered neutrophils and extensive hyalinization, karyolysis, cytolysis and vacuolation of the parenchyma. The submarginal layers of neutrophils were narrow, indistinct or absent. The congested border of the necrotic area adjoining the viable cortex was occupied by closely packed lipoid-laden large mononuclear cells of the epithelioid type (described more fully later) forming a layer ranging up to 200 microns in diameter. These epithelioid cells were often separated into columns by radiating slender septal downgrowths of swollen endothelium and fusiform fibroblasts. The congested border of the necrotic area adjoining the medulla often showed extensive extravasation of red blood cells and, in some animals, slight to marked proliferation of fusiform fibroblasts grading into a thin layer of fibroblastic connective tissue encircling the medulla. Many of these fusiform cells were laden with lipoid material, and, in some animals, with pigment giving the prussian blue reaction for iron (hemosiderin). In one adrenal gland the connective tissue layer was focally contiguous with the layer of epithelioid cells.

Guinea pigs killed nine, eleven, fourteen and sixteen days after one exposure showed progressive thickening of the adrenal cortex, extensive vacuolation and progressive reduction in size of the necrotic area, and gradual thinning of the layer of epithelioid cells, which often became continuous with the connective tissue layer as already described. Of 3 killed twenty-one days after a single exposure, the layer of epithelioid cells was present in only 1, and the necrotic area had completely disappeared in all. The connective tissue layer about the medulla showed much fat and hemosiderin.

Mitotic figures occurred chiefly in the zona fasciculata, particularly near the necrotic border. They were absent or rarely seen in the cortex of any control animal or any exposed animal killed immediately or more than nine days after one exposure to dichloropropane. In single microscopic cross sections of the adrenal glands of individual animals, mitotic figures numbered 0 to 4, 2 to 29 and 3 to 36 in groups of guinea pigs killed immediately after two, three and four exposures, respectively. In experiment B they numbered from 3 to over 300 per microscopic section in guinea pigs killed one and two days after a single exposure, and 6 to 20, 0 to 10 and 0 to 2 in those killed four, seven and nine days, respectively, after the single exposure.

In line with previous observations,⁵ our unexposed controls showed only a small amount of lipoid material in the zona glomerulosa and abundance of lipoid material occurring as fairly uniform fine droplets confined chiefly to the outer half of the zona fasciculata. Most guinea pigs killed immediately after two to four exposures and a lesser number of those killed sixteen hours to four days after a single exposure showed a slight to moderate increase of lipoid material in the zona glomerulosa. Except for the layer of cells bordering the necrotic area, the viable portion of the zona fasciculata often showed a slight decrease of lipoid material with a relative increase of the number of coarser globules. The necrotic area showed many coarse lipoid globules, and as the length of time after a single exposure or the total number of exposures increased there was generally a corresponding decrease in the amount of fat in the necrotic parenchymal cells and an increase of the amount in the infiltrating neutrophils and mononuclear cells.

In general, the epithelioid cells in the necrotic area were sharply delineated and readily distinguished from the adjacent regenerating cortical cells. The latter appeared fairly uniform in size and shape with a centrally placed large spherical nucleus and abundant, usually lightly eosinophilic cytoplasm filled with small lipoid

5 Jaffe, R. Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere, Berlin, Julius Springer, 1931.

globules In contrast, the epithelioid cells in many areas varied markedly in size and shape Their nuclei were round, ovoid, elongate or irregular in shape, often eccentric and generally more deeply stained, with less eosinophilic karyoplasm, than those in the adjacent cortex The cytoplasm of the epithelioid cells was poorly stained and contained few to many unevenly spaced lipid globules of variable size and some nonlipoid vacuoles Even when mitotic figures were numerous in the regenerating cortex, none were seen in the subjacent epithelioid cells

The adrenal glands of guinea pigs killed immediately after a single seven hour exposure to 2,300 ppm of dichloropropane showed slight to marked congestion and edema of the medulla In animals killed after two to four daily exposures and sixteen to forty-eight hours after one exposure the medulla showed in addition extensive hemorrhagic extravasations, lymphocytic and neutrophilic infiltration and disorganization of the nests of chromaffin cells The cytoplasm of these cells was often vacuolated, and their nuclei were occasionally pyknotic, poorly stained or fragmented. A few cells were necrotic, many were atrophic, and there appeared to be a slight to marked reduction in their numbers Fine fat droplets were seen occasionally in some chromaffin cells as well as in isolated cortical cells in the medulla The amount of chromaffin pigment was often reduced An occasional mitotic figure was seen in the chromaffin cells of 1 guinea pig killed after three exposures and in 1 after four exposures

Most animals killed four and seven days after exposure showed less extensive hemorrhage and some proliferation of fibroblasts and deposition of hemosiderin in the medulla Most animals killed nine days or more after one exposure showed little or no hemorrhage, a restoration of normal structure and amount of parenchyma, a decrease of infiltrating cells and a slight increase of hemosiderin.

MICROSCOPIC CHANGES IN RATS

Specific lesions were found in the livers, the kidneys and the adrenal glands

In experiment A (repeated exposures) no significant changes were seen in the livers of rats exposed for only four hours One of 3 rats killed after one seven hour exposure showed slight, diffuse, fine droplet fatty degeneration of the liver Five rats killed after two exposures showed marked diffuse fatty degeneration, 4 of them showed also extensive, often confluent centrilobular necrosis involving more than half the parenchyma Some lobules showed little or no involvement, while others were largely or completely necrotic In the necrotic areas, the liver cords were often narrowed, distorted or disorganized, the liver cells were poorly outlined, their cytoplasm was deeply eosinophil, with occasionally basophil stippling, and their nuclei were often inapparent, poorly stained, pyknotic or fragmented Kupffer cells showed less definite changes The sinusoids in the necrotic areas were often narrowed and distorted and contained variable numbers and proportions of red blood cells, neutrophils and pyknotic and fragmented nuclei and nuclear debris There were also some lymphocytes and occasional larger polyhedral or stellate cells with deeply staining nuclei and a small amount of cytoplasm, often containing some fine lipid globules Some necrotic areas showed foci of hemorrhage, and many showed a narrow peripheral hemorrhagic zone

One of the 5 rats killed after three exposures showed a more advanced similar necrosis and narrowing of the liver cell cords Another showed subtotal replacement of the central third of the hepatic lobule by closely packed red blood cells and fat-laden mononuclear cells similar to those already described Few Kupffer cells, lymphocytes and occasional necrotic liver cells remained, chiefly near the midzone There was a moderately broad midzonal area of fatty degeneration bordering the necrotic area The other 3 rats showed intermediate changes Rats

killed after four exposures showed a narrow centrilobular cellular zone composed chiefly of monocytes with few or no evident necrotic cells and surrounded by a narrow zone of fatty degeneration. Rats killed after five exposures showed either no change or only a few centrilobular lymphoid cells and monocytes with a few marginal fat-laden liver cells. Numerous mitotic figures were seen only in 1 of 5 rats killed after three exposures.

In experiment B no significant changes were seen in the livers of rats killed immediately and seven days or more after the single seven hour exposure to 2,200 ppm of 1,2-dichloropropane. The 5 rats killed twenty-four hours after the exposure showed moderate to marked, midzonal to diffuse, fine droplet fatty degeneration of the liver and centrilobular necrosis. The latter was similar to but more sharply demarcated and less extensive and less hemorrhagic than that observed in the rats killed immediately after the two exposures. The hepatic lesions in rats killed two and four days after the single exposure were essentially similar to those seen in rats killed immediately after four and five daily exposures, respectively. Only 3 of 5 rats killed two days and 1 of 3 killed four days after the single exposure to dichloropropane showed some necrotic liver cells in a few of the centrilobular lesions. Only the livers of rats killed two days after one exposure showed a few to moderately numerous scattered mitotic figures. Most rats killed one and two days after the single exposure showed marked depletion of glycogen in the liver. Most rats killed four days or more after the single exposure to dichloropropane showed hemosiderin in some Kupffer cells, chiefly centrilobularly.

In the kidney, fine droplets of fat occurred chiefly in the epithelium of many convoluted tubules and thick portions of the loops of Henle. In experiment A a small amount of renal fat was seen in 3 of 6 rats killed after two daily exposures, in 4 of 5 after three, in 1 of 5 after four and in none of 5 after five exposures. In experiment B a small amount of renal fat was seen only in 4 of 5 rats killed one day and 2 of 5 killed two days after a single exposure, a minimal amount was seen in the kidneys of 3 of 5 rats killed seven days after exposure.

The fat in the adrenal cortex was moderately reduced in rats killed at once after a four hour exposure to 2,200 ppm of dichloropropane, was markedly reduced after one and two seven hour exposures, slightly reduced after three and within normal limits after four and five such exposures. In experiment B the amount of fat in the cortex was slightly to moderately reduced only in rats killed at once and twenty-four hours after a single exposure.

In experiment A hemosiderin in small amounts was seen in the spleen only in rats killed after five exposures and in 3 of 5 rats killed after four exposures. In experiment B hemosiderin was seen in both controls and exposed animals in variable amounts.

Other changes included slight bronchitis seen in 6 rats killed after three exposures, 1 of these showed in addition slight interstitial pneumonitis, and 1 showed a seromonocytic lobular pneumonia. In experiment B interstitial pneumonitis was seen in occasional animals in nearly all groups, including the controls.

COMMENT

As is well known, intermittently exposed laboratory animals may develop increased resistance to certain toxic substances quite rapidly.⁶ It would appear that 1,2-dichloropropane is such a substance. Thus, clinically we have previously noted^{2a} that the first few exposures to dichloropropane cause marked narcosis, whereas subsequent exposures have no

apparent narcotic effect on the animals. The present investigation shows that this increasing resistance is reflected also in the pathologic picture. Thus, when we compare the results in experiments A and B, we find in general that the lesions occurring during a series of daily exposures to dichloropropane are similar to those following a single exposure and that such lesions, though somewhat more severe and prolonged, apparently undergo rapid resolution despite continuation of daily exposures.

The rapidity of development and the apparent complete resolution of such lesions can be illustrated by giving a brief composite picture of the changes observed in the livers of exposed rats in experiment A. Thus, we found no significant hepatic changes immediately after one four-hour exposure to 2,200 p p m of 1,2-dichloropropane, slight diffuse fatty degeneration after one seven-hour exposure, marked diffuse fatty degeneration and extensive, often confluent centrilobular coagulation and focal hemorrhagic necrosis after two such exposures, centrilobular congestion and cellular infiltration with occasional necrotic liver cells and midzonal fatty degeneration after three exposures, slight centrilobular lymphocytic and monocytic infiltration and midzonal fatty degeneration after four exposures and minimal or insignificant changes after five such exposures to dichloropropane. Similar rapid changes were seen in other organs.

In general, the results of the present experiment are in keeping with those reported in our first paper^{2a} in a few similarly exposed animals. In the earlier experiments a few guinea pigs that died showed extensive centrilobular coagulation necrosis similar to that seen in the rats. Since the animals that died in earlier experiments often showed more severe lesions than similarly exposed killed animals, it seems not unlikely that dichloropropane can produce such extensive hepatic necrosis only in some of the more susceptible guinea pigs and that it may have produced some of the small hepatic foci of coagulation necrosis in the exposed guinea pigs in our present experiments, even though similar foci were seen occasionally in control animals.

In the earlier experiments, only occasional minimal lesions were noted in animals surviving many exposures to dichloropropane. In the light of the present experiments it seems probable that severe lesions were produced initially in many or all of these much exposed animals and that such lesions underwent early resolution despite further exposures, leaving little or no trace of their former presence. Thus, in our earlier experiments the adrenal glands of guinea pigs killed after surviving many exposures showed a subcortical layer of connective tissue. The observations in our present experiments suggest that this connective tissue layer may have replaced an earlier area of hemorrhagic necrosis at the border of the cortex and the medulla.

Lesions in the adrenal glands of guinea pigs somewhat similar to those noted in our experiments with dichloropropane have been reported

to follow the administration of carbon tetrachloride⁷ Phelps and Hu⁸ reported that necrosis of the adrenal cortex following subcutaneous injection of carbon tetrachloride appeared later and disappeared earlier than the lesions in the liver. In contrast, our experiments show that dichloropropane can produce severe lesions in both the cortex and the medulla of the adrenal gland of the guinea pig before lesions in the liver become manifest. It seems not unlikely, therefore, that in the guinea pig the damage to the adrenal gland may be more important than that to the liver in accounting for the mortality and the toxicity of dichloropropane. In view of these results in guinea pigs, perhaps the depletion of lipid material and other slight changes found at times in the adrenal glands of rats and other animals exposed to dichloropropane and some other compounds⁹ should merit more attention from a toxicologic standpoint.

SUMMARY

Two experimental groups, each made up of guinea pigs and rats, were exposed to 2,200 p.p.m. of 1,2-dichloropropane. In one experiment the animals were killed for histologic study at intervals during a short series of daily exposures, in the other experiment they were killed at intervals following one seven-hour exposure.

Guinea pigs showed extensive coagulation and focal hemorrhagic necrosis of the adrenal cortex, congestion with hemorrhage and parenchymatous degeneration of the adrenal medulla, and fatty degeneration of the liver and the kidney. Rats showed hepatic centrilobular necrosis, fatty degeneration of the liver and the kidney, and depletion of the lipid material of the adrenal cortex. In both experiments these lesions did not reach their full development generally until twenty-four to forty-eight hours after the end of the first exposure. These lesions were somewhat less severe and disappeared earlier in animals receiving one exposure than in those receiving daily exposures. However, except for the adrenal glands of guinea pigs, lesions were minimal or absent three or four days after the end of the first exposure even in the animals that continued to receive daily exposures. The necrotic area in the adrenal cortex did not disappear completely until two to three weeks after a single exposure.

These observations emphasize the importance of obtaining sufficient pathologic material early in a numerical series of intermittent exposures to toxic agents in order to determine the frequency, the character, the development and the resolution of any lesions produced.

7 Smyth, H. F., Smyth, H. F., Jr., and Carpenter, C. P. *J. Indust. Hyg. & Toxicol.* **18**: 277, 1936.

8 Phelps, B. M., and Hu, C. H. *J. A. M. A.* **82**: 1254, 1924.

9 Heppel, L. A., Neal, P. A., Daft, F. S., Endicott, K. M., Orr, M. L., and Porterfield, V. T. *J. Indust. Hyg. & Toxicol.* **27**: 15, 1945.

FIBRINOGEN PLASTICS

Tissue Reactions Induced by a Series of Fibrinogen Plastics Implanted in the Abdominal Wall of Guinea Pigs

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AND

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THE NEW METHODS developed for the fractionation of human blood plasma have made it possible to prepare the constituent proteins in large amounts and in a high state of purity¹. Several of the plasma proteins, notably fibrinogen, may be made into plastic materials². The physical properties of these plastics vary greatly, depending on the plasticizer used as well as on the temperature and the pressure at which plasticization takes place. These properties have been described in detail elsewhere².

In recent years a number of publications have appeared which deal directly or indirectly with the tissue reactions elicited by commercial plastics implanted in living tissue. Although many of these plastic materials have very different properties when the conditions of formation are altered, the investigations mentioned have largely been confined to unmodified commercial products. Since the fibrinogen plastics are easily made under controlled conditions in a research laboratory, it is worth while to study the tissue reactions induced by a series of modified plastics. In this way, it may be possible to characterize the

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This paper is No. 49 in the series "Studies on Plasma Proteins" from the Harvard Medical School, Boston, on products developed by the Department of Physical Chemistry from blood collected by the American Red Cross

The work described in this paper has been carried out in part under a grant from the Proctor Fund of Harvard University and in part under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University

1 Cohn, E. J., Oncley, J. L., Strong, L. E., Hughes, W. J., Jr., and Armstrong, S. H., Jr. *J. Clin. Investigation* **23** 417, 1944. Edsall, J. T., Ferry, R. M., and Armstrong, S. H., Jr. *ibid.* **23** 557, 1944. Cohn, E. J., Strong, L. E., Hughes, W. L., Jr., Mulford, D. J., Ashworth, J. N., Meln, M., and Taylor, H. L. *J. Am. Chem. Soc.* **68** 459, 1946.

2 Ferry, J. D., and Morrison, P. R. *J. Clin. Investigation* **23** 566, 1944. Ferry, J. D., and Morrison, P. R. *Fibrin Film and Other Plastic Products from Human Plasma*, to be published.

variations in tissue reactions to fibrinogen plastics due to several variables in composition. Furthermore, the study of this series may serve as a basis for comparison with studies of other groups of plastics used in medicine. This study was originally undertaken to determine whether or not fibrinogen plastics were suitable for use in surgical practice. The results, together with certain other material, were included in a report to the Proctor Fund, Sept 1, 1942 ("The Behavior of a Series of Fibrinogen Plastics in Animal Tissues, with a Note on the Effect of Plasticizing Agents on Fibrinogen-Thrombin Films" by O. T. Bailey, R. Ford, and C. V. Z. Hawn).

METHODS FOR THE STUDY OF TISSUE REACTIONS TO FIBRINOGEN PLASTICS

A standard procedure was set up for the study of the series of fibrinogen plastics. The plastics to be tested were prepared under sterile conditions. Guinea pigs weighing 350 to 450 Gm were used. The animal was anesthetized with ether and the abdomen shaved. A small incision was made in the abdominal skin and carried down to the deep fascia. A piece of plastic approximately 0.7 by 0.4 by 0.4 cm was inserted into the fascial layer between the skin and the musculature, it was then pushed lateralward so that the plastic did not lie beneath the incision. The incision was then closed with one black silk suture. Aseptic technic was used for the operative procedure, but no dressing was applied. Four to six pieces, differing from one another in composition, were implanted in different portions of the abdominal wall of each animal. The animals were killed with chloroform at various intervals. The plastics and the tissues surrounding them were immediately removed and fixed in Zenker's fluid containing glacial acetic acid. After fixation, the tissues were embedded in paraffin, and sections were cut and stained by the eosin-methylene blue technic and occasionally by the hematoxylin-eosin method. The plastics tended to become tough in the graded alcohols used for dehydration of the tissue blocks. When this change was sufficient to preclude the cutting of satisfactory paraffin sections, the plastic was gently removed from the tissue block before embedding. The surrounding tissue was not adherent to the plastic until the plastic was almost completely absorbed.

EFFECT OF VARYING THE PLASTICIZER

A series of plastics was prepared with different plasticizers, other variables being kept constant. In each instance, the substrate was bovine fibrinogen, the pressure 120 pounds per square inch (8.5 Kg per square centimeter), the temperature 100 C and the length of time in the hot press thirty minutes. Equal parts of plasticizer and bovine fibrinogen were used (except in the tenth instance in the following list).

The plasticizers used were

- | | |
|--|--|
| 1 Glycerin | 6 Ethylene glycol monoacetate |
| 2 Glycerin plus 5 per cent thiocyanate | 7 Tetraethylene glycol |
| 3 Ethylene glycol | 8 Water |
| 4 Ethylene glycol plus 5 per cent sodium thiocyanate | 9 Water plus 5 per cent sodium thiocyanate |
| 5 Propylene glycol | 10 No plasticizer |

The cellular responses to these plastics and to the plastics described in other sections of this report showed such great qualitative similarity that a single

description will suffice. The physical characteristics of the materials, their persistence in the tissues and the quantitative tissue reactions varied considerably and will be indicated for each of the plastics tested.

The initial cellular response consisted of slight cellular infiltration—polymorphonuclear leukocytes, followed by mononuclear cells and lymphocytes. The number of all these cells gradually increased somewhat, at the same time there was slight proliferation of fibroblasts with laying down of collagen. A few giant cells were formed along the edge of some of the plastics, but giant cells at this stage appeared in surprisingly small numbers or not at all. It was difficult to determine how much of the initial cellular reaction resulted from the trauma of inserting the plastic and how much from the irritation caused by the presence of the



Fig 1—Tissue reaction to plastic made from bovine fibrinogen with glycerin as plasticizer (each 50 per cent) at 100 C for two hours. Animal killed eight weeks after implantation. Hematoxylin-eosin stain, low magnification, plastic removed. The tissue closest to the plastic is at the lower margin of the photomicrograph.

implant. The giant cells were almost certainly the result of the presence of the plastic, while some of the polymorphonuclear leukocytes and mononuclear cells might well have appeared in response to the trauma.

Regardless of plasticizer, the plastics stained blue with eosin-methylene blue technic but took the eosin stain when the hematoxylin-eosin method was used. In the eosin-methylene blue sections they soon became eosinophilic along the line of contact with living tissue. As absorption progressed, the portion of plastic in contact with living tissue remained more eosinophilic than that at a distance

from living tissue. This staining change was accompanied by infiltration of many mononuclear cells, which contained eosinophilic material histologically identical with the surrounding plastic and judged to be phagocytosed bits of the material. Polymorphonuclear neutrophils made their appearance in the region of disintegration of the plastic. The mononuclear cells containing eosinophilic material became scattered through the connective tissue about the plastic as though they were moving away from the zone of phagocytosis. Scattered eosinophils and mononuclear cells were also found in this connective tissue. Small pieces were broken off at the edge of the plastic, these became surrounded by mononuclear cells and polymorphonuclear neutrophils. About some of them, giant cells were formed even at this stage, however, giant cells were not a dominant

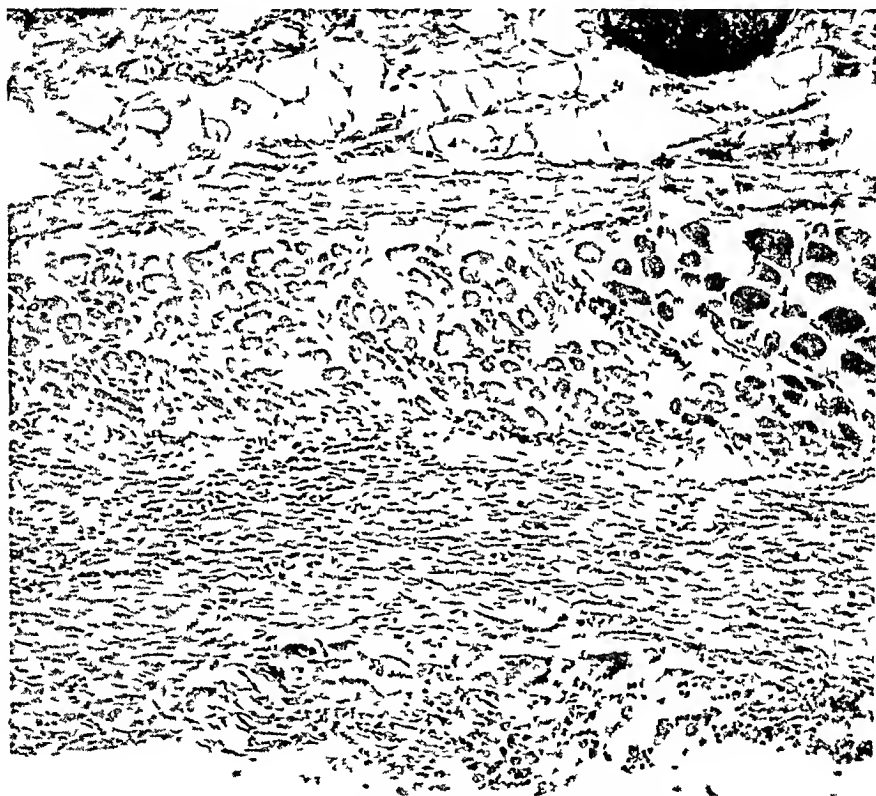


Fig 2—Tissue reaction to plastic made from bovine fibrinogen with water as plasticizer (each 50 per cent) at 100 C for thirty minutes. Animal killed four weeks after implantation. Hematoxylin-eosin stain, low magnification, plastic removed. The tissue closest to the plastic is at the lower margin of the photomicrograph.

feature of the cellular response. The next step in disintegration was indicated in microscopic sections by the appearance within the plastic of a few widely separated strands of connective tissue, accompanied by small numbers of mononuclear cells and polymorphonuclear neutrophils. These strands extended all the way through the plastic at various angles. The mechanism concerned in their abrupt appearance was not determined from the microscopic sections. Complete disintegration of the plastic soon followed. During this process the polymorphonuclear neutrophils and mononuclear cells increased in number. While there

was active phagocytosis, there did not seem to be sufficient to account for the removal of all or even the larger part by this process alone. While there is no direct evidence, it seems probable that enzymatic activity played a large part in the disappearance of the material. The rapid completion of removal was judged to be due to the fact that living cells and tissue fluids had free access to all parts of the plastic after the ingrowth of the widely separated strands of tissue. The end result was a small fibrous cicatrix, identified with difficulty and often not found even when sections were cut from serial blocks of the entire region.

In spite of the qualitative similarity of the tissue reactions to plastics made with different plasticizers, there was considerable quantitative variation in the responses. The least reaction was obtained when ethylene glycol was the plasticizer. Plastics made with glycerin gave little more (fig 1). Those made with water gave considerably more reaction (fig 2) (there was one exception to this in the

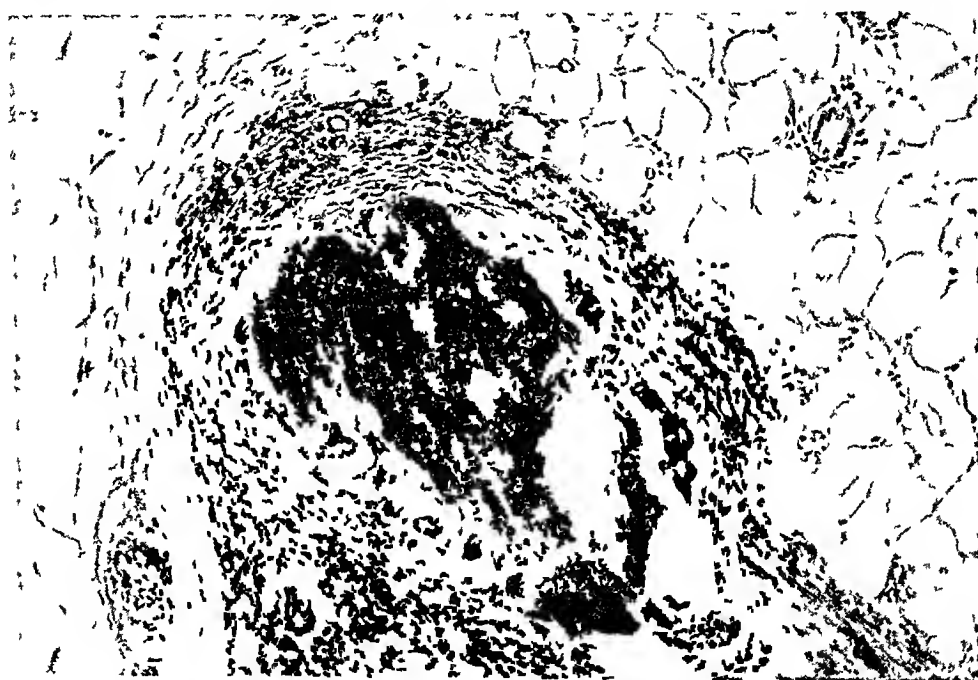


Fig 3—Tissue reaction to plastic made with ethylene glycol twenty-five weeks after implantation. Hematoxylin-eosin stain, low magnification.

series). The other specimens produced reactions which were intermediate between those produced by ethylene glycol plastics and those produced by water plastics. The plastic made of fibrinogen without plasticizer produced about the same reaction as the intermediate group of plastics. The addition of sodium thiocyanate had a variable effect on the amount of tissue reaction. In some cases the amount of reaction was unchanged, while in others it was increased. In no instance was the reaction diminished by the addition of sodium thiocyanate.

The glycerin plastics disappeared in six weeks to three months. The propylene glycol and ethylene glycol plastics survived in the tissues, being present in animals examined as long as six months after implantation (fig 3). Plastics made with water disintegrated at about the same rate as those made with glycerin. The others occupied an intermediate position. The addition of sodium thiocyanate had no significant effect on the rate of disintegration of the materials.

EFFECT OF VARYING THE CONCENTRATION OF PROTEIN IN THE PLASTIC

A series of plastics was prepared with varying concentrations of bovine fibrinogen. All were heated at 100 C at a pressure of 120 pounds per square inch for thirty minutes. The following variables were used in preparing the series of plastics:

- 1 Water 33 per cent—fibrinogen 67 per cent (tough, rubbery)
- 2 Water 67 per cent—fibrinogen 33 per cent (opaque, soft)
- 3 Water 80 per cent—fibrinogen 20 per cent (soft)
- 4 Water 91 per cent—fibrinogen 9 per cent (separated into clot and aqueous phases)
- 5 Ethylene glycol 33 per cent—fibrinogen 67 per cent (tough, rubbery)
- 6 Ethylene glycol 67 per cent—fibrinogen 33 per cent (soft)
- 7 Ethylene glycol 80 per cent—fibrinogen 20 per cent (weak, slightly opaque)
- 8 Glycerin 33 per cent—fibrinogen 67 per cent (tough, rubbery)
- 9 Glycerin 67 per cent—fibrinogen 33 per cent (soft)
- 10 Glycerin 80 per cent—fibrinogen 20 per cent (slightly opaque, weak)
- 11 Glycerin 10 per cent plus sodium thiocyanate 80 per cent—fibrinogen 20 per cent (clear, weak)
- 12 Glycerin plus 10 per cent sodium thiocyanate 91 per cent—fibrinogen 9 per cent (clear, very soft)
- 13 No plasticizer—fibrinogen 100 per cent (stiff, brittle)

Plastics containing 50 per cent plasticizer and 50 per cent protein were available from the previous series of experiments.

The difference in concentration of the protein had no effect on the character of the cellular response. Plastics containing 80 and 91 per cent water underwent almost complete disintegration in the tissues within three weeks. In other instances the persistence of the plastic was affected little by the change in concentration of the protein in spite of the alterations in physical properties due to the variations in composition.

EFFECT OF VARYING THE TIME OF HEATING

A series of plastics was prepared to test the effect of varying the length of the period during which the material was heated at 100 C under 120 pounds' pressure per square inch. Equal parts of bovine fibrinogen and plasticizer were used in each instance. The plasticizers used and the periods of heating were as follows:

| | |
|------------|---------|
| 1 Water | 5 min |
| 2 Water | 15 min |
| 3 Water | 30 min |
| 4 Water | 120 min |
| 5 Glycerin | 5 min |
| 6 Glycerin | 15 min |
| 7 Glycerin | 30 min |
| 8 Glycerin | 120 min |

As the time of heating was lengthened, the plastics became darker brown, firmer and less flexible. No significant differences in tissue reaction to the plastics made with water resulted from the variation in the time of heating. In the case of the glycerin plastics the reaction was considerably greater at five minutes and at one hundred and twenty minutes than it was at fifteen minutes or thirty minutes. From these experiments we were unable to draw any general conclusions on the relation of the tissue reaction to the length of time the material was heated.

EFFECT OF VARYING THE PROTEIN USED AS SUBSTRATE

Each of the substrates used was mixed with an equal amount of water. The mixture was heated at 100 C under a pressure of 120 pounds per square inch for thirty minutes. The substrates tested were

- 1 Bovine albumin
- 2 Horse fibrin (Horse Fiber Powder)
- 3 Human fibrinogen
- 4 Casein
- 5 Bovine fibrinogen

The plastic made with bovine albumin produced a minimal tissue reaction, comparable to that produced by plastics composed of ethylene glycol and fibrinogen. When horse fibrin was used as the substrate, a severe tissue reaction was elicited, more extensive than that to any other plastic tested in the entire study. The commercial horse fibrin contained a much higher percentage of impurities than the bovine fibrinogen, which may account for some of the severity of the tissue reaction. Plastics with human fibrinogen and casein as substrates produced reactions similar to those appearing in response to plastics with bovine fibrinogen as substrate, described in previous sections of this report. No experiments with plastics made from homologous fibrinogen were carried out on animals.

COMMENT

The purpose in undertaking this study was to determine which of the plastics was most suitable for clinical trial. As experience with the fractionation of human and bovine plasma increased, Ferry and Morrison² and Bering³ were able to produce from fibrinogen and thrombin other materials which suited the immediate problems of the armed forces better than did the plastics. These received clinical trial and were used in large amounts in the armed forces.⁴ There may still be uses for the fibrinogen plastics in human surgery, as indicated by Blaine.⁵ Should one of these plastics be required for human patients, the data recorded in this paper could be used in the selection of one with a suitable time of persistence and character of tissue reaction. The plastics tend to soften in the tissues long before they are completely absorbed.

In a series of experiments not recorded in detail, plastics were prepared with mixed plasticizers (e g, fibrinogen 50 per cent, ethylene glycol 25 per cent, water 25 per cent). These gave tissue reactions resembling those in response to plastics made with the more reactive single plasticizer (in the example cited, a water-fibrinogen plastic). When any of these plastics is sterilized in steam, a considerable amount of the plasticizer is replaced by water, and the tissue reaction should be expected to be altered somewhat.

3 Bering, E A, Jr. *J Clin Investigation* **23** 586, 1944

4 Ingraham, F D, and Bailey, O T. *J Neurosurg* **1** 23, 1944. Bailey, O T, and Ingraham, F D. *J Clin Investigation* **23** 591, 1944, **23** 597, 1944. Ingraham, F D, and Bailey, O T. *J A M A* **126** 680, 1944. Ingraham, F D, Bailey, O T, and Cobb, C A, Jr. *ibid* **128** 1088, 1945.

5 Blaine, G. *Brit J Surg* **33** 245, 1946

When various plastic materials, e g nylon (a concentration product of hexamethylene diamine and adipic acid) and lucite (methyl methacrylate), have been used in surgical practice, the investigators have usually been content to employ the unmodified commercial product. By trying a series of related plastics, as has been done for the fibrinogen plastics, one or several might be found especially suited for particular surgical needs.

SUMMARY

A series of plastics was prepared from various purified blood proteins, especially fibrinogen. Their physical properties could be varied over a wide range by altering the substrate, the plasticizer and the conditions of plasticization.

Studies were made to determine the tissue reactions elicited by a group of these plastics in the abdominal wall of guinea pigs. On the whole, the tissue reactions were relatively slight. The tissue reactions were affected by the nature of the substrate and of the plasticizer, they were much less affected by the concentration of the substrate and by the time of heating.

Case Reports

PRIMARY BRONCHIOGENIC LEIOMYOSARCOMA

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AND

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MESENCHYMAL tumors of bronchial origin are extremely rare. The most frequently reported of this group of mesenchymal lesions are the chondromatous tumors. Of tumors arising from smooth muscle and limited to the bronchus, 6 have been previously recorded.

Franco¹ reported the incidental finding of a tumor arising from the right upper lobe bronchus in a 56 year old woman who died of meningitis. The tumor was ovoid and circumscribed, measuring 13 by 11 by 9 cm and histologically was a leiomyoma. In his report he cited 2 previous cases, one recorded by Forkel² and the other by Deussing³. In each of these 2 cases the tumor was found at autopsy, in Forkel's case it was solitary, while in Deussing's case it occurred as multiple bilateral pulmonary lesions, which suggested to some reviewers metastasis from similar tumors found in the patient's uterus.

Neumann⁴ described a cancerous tumor of smooth muscle arising in the left upper lobe bronchus of a 66 year old woman, which metastasized throughout each lung and to the thyroid gland, the liver, the pancreas, the adrenal glands and the lumbar vertebrae.

Kramer⁵ reported a case. A 15 year old girl complained of cough with recurrent blood-streaked sputum and clubbing of the fingers. Roentgen examination revealed a triangular shadow in the mesial portion of the lower lobe of the right lung. Bronchoscopy revealed partial occlusion of the bronchus of this lobe by a broad-based pedunculated tumor, diagnosed histologically as myoblastoma.

Brahdy⁶ reported still another case. An 18 year old nurse, while under observation for early pulmonary tuberculosis, was found by roentgen examination to have a circumscribed tumor in the lower two thirds of the lower lobe of the right lung. This was successfully removed and a diagnosis of fibroleiomyoma made on microscopic examination.

From the Pathology Section of the Laboratory Service and the Thoracic Surgery Section of the Surgical Service, Walter Reed General Hospital, Washington, D. C., and the Department of Surgery, George Washington School of Medicine, Washington, D. C.

1 Franco, E. E. *Tumors* 3:27, 1929

2 Forkel, W. *Ztschr f Krebsforsch* 8:390, 1909

3 Deussing, R. *Multiple primäre Myome der Lunge*, Inaug. Dissert., Munich, R. Muller & Steinicke, 1912

4 Neumann, R. *Frankfurt Ztschr f Path* 52:576, 1938

5 Kramer, R. *Ann Otol, Rhin & Laryng* 48:1083, 1939

6 Brahdy, L. *Am Rev Tuberc* 43:429, 1941

The tumor reported here is the seventh of such mesenchymal tumors recorded in the literature

REPORT OF A CASE

A well nourished Negro man aged 34 was admitted to Walter Reed General Hospital in August 1945 with chronic cough, dyspnea and weakness. The persistent cough began in the early part of 1943, however, hospitalization was not necessary during that period, and no roentgen examination of the chest was made. In November 1944, while at a high altitude overseas, the patient had hemoptysis of about 2 ounces (59 cc). In February 1945 he was admitted to a hospital, at which time the cough was productive of thick, greenish yellow sputum and a diagnosis of "pneumonia, right lung," was made. There was persistent leukocytosis, and the sedimentation rate was 28 mm in one hour. Roentgen examination of the chest revealed an area of density in the right hilar region, with a shift of the heart and the mediastinum to the right and an elevation of the right leaf of the diaphragm.

The patient was transferred to the Zone of the Interior, and in June 1945 there was roentgen evidence of appreciable enlargement of the bronchial neoplasm. Bronchoscopy at this time revealed a smooth, rounded tumor almost completely occluding the right main bronchus and partially covering the coryna. Repeated bronchoscopic resections of the tumor resulted in transient reexpansion of the right lung, with the mediastinum shifting back to a more normal position. The tumor during these periods was visualized as a well circumscribed mass in the right main and right upper lobe bronchi.

As irradiation therapy was thought advisable, the patient was transferred to Walter Reed General Hospital with a diagnosis of bronchiogenic neoplasm, type undetermined. During bronchoscopy at this hospital considerable bleeding was encountered, this being controlled by sponges saturated with solution of epinephrine hydrochloride. A biopsy specimen was taken at this time and a diagnosis of low grade leiomyosarcoma made (fig 1 B).

During this hospitalization the patient had hemoptysis on several occasions, varying in amounts up to 400 cc. Because of the location and the type of the tumor, with involvement of the coryna and repeated episodes of hemoptysis, pneumonectomy was considered the only possible method of prolonging the patient's life. This was attempted on October 15. As the right main bronchus was being exposed, the portion of the tumor at the coryna became detached so that it completely obstructed the air passages, this obstruction was relieved by immediate bronchoscopy. It was decided to complete the operation in a second stage at a later date. However, during closure of the chest there was profuse bleeding into the left main bronchus and all efforts of resuscitation were of no avail.

Postmortem Examination—Externally the body revealed no abnormalities except for the wound left by the recent thoracotomy with resection of the right fifth rib. The right pleural cavity contained approximately 100 cc of fresh blood and no adhesions. The left pleural cavity was free of fluid and adhesions, while the lung was partially collapsed. The peritoneal cavity contained a normal amount of clear, thin fluid. The abdominal viscera were in their normal position and revealed no abnormalities.

The lungs, the trachea and the larynx were removed en masse. The right lung was dark purple and the surface smooth and glistening. A circumscribed mass was palpable in the right hilar region, extending into the upper lobe of the lung. The trachea and bronchi were opened posteriorly, revealing a friable mass 2 cm

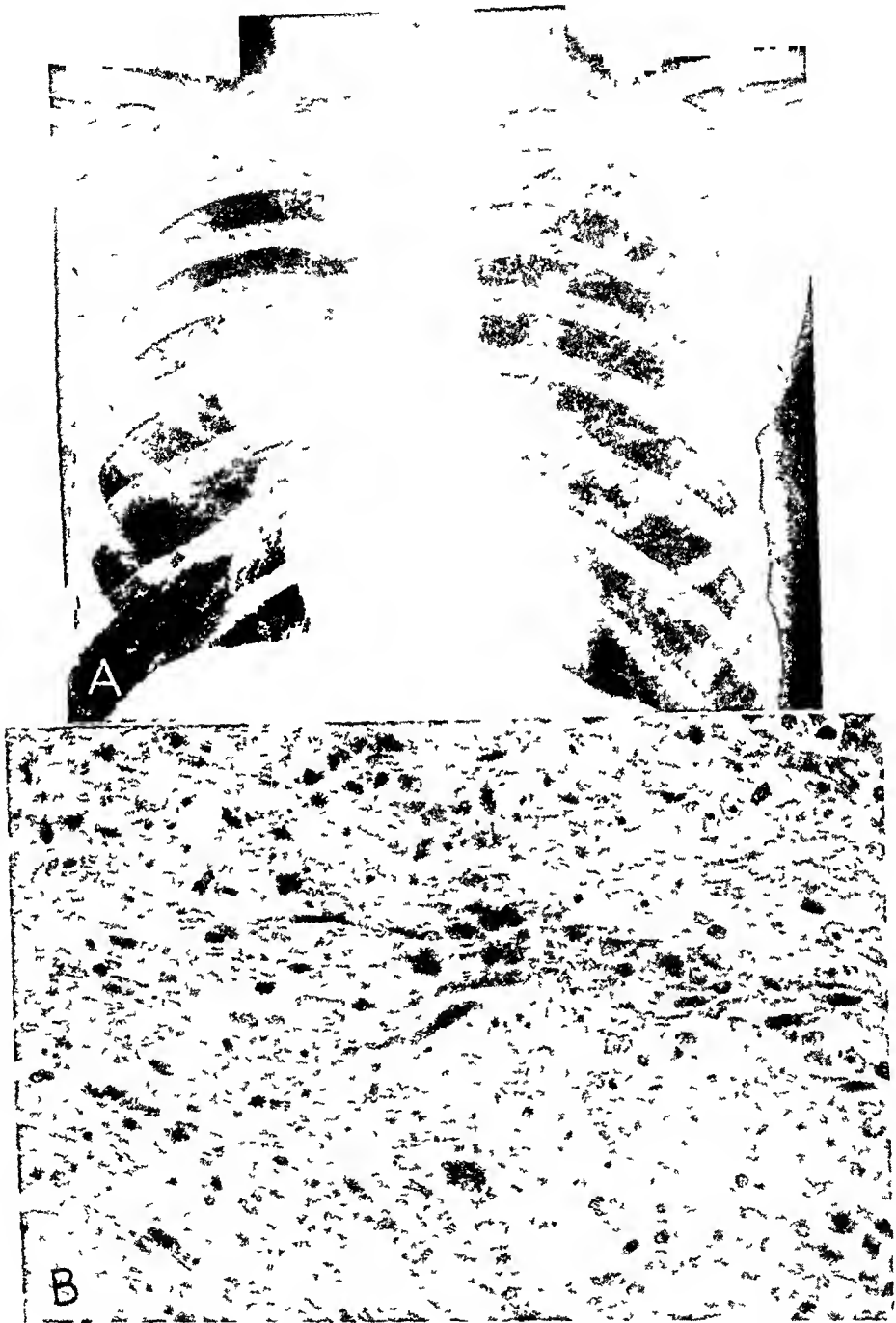


Fig 1—*A*, roentgenogram taken in August 1945 after bronchoscopic resection of the tumor attached to the coryna. It shows the lung almost completely expanded. The neoplasm appears essentially the same as when visualized two months previously.

B, photomicrograph of a section of the bronchoscopic biopsy specimen. Note the complete absence of epithelial structures, the fragment being composed of compact ovoid and spindle-shaped smooth muscle cells. $\times 400$ (United States Army Medical Museum Neg 94799)

in diameter, attached to the coryna by a pedicle approximately 3 mm in thickness and length. Detached from this portion was the large friable hemorrhagic tumor enclosed within the right main bronchus proximally, but extending along the upper lobe bronchus, where the adjacent lung parenchyma was infiltrated. The left main bronchus was completely filled with fresh blood and clots.



Fig. 2—Upper and middle lobes of the lung containing the tumor sectioned after fixation. The tongue-like projection represents the middle lobe, entirely free from tumor. Note the sharp line of demarcation between the tumor and the surrounding hemorrhagic lung parenchyma.

When the lung was sectioned, the tumor was found to be irregularly circumscribed, 10 to 14 cm in diameter, soft, yellowish to hemorrhagic and limited to the upper lobe. The surrounding parenchyma was atelectatic and hemorrhagic (fig 2).

The heart and the abdominal viscera were removed, and careful gross and microscopic examination revealed no evidence of tumor or other significant abnormality.

Microscopic Examination—Sections of the tumor removed at autopsy presented the typical appearance of an extremely cellular but locally infiltrative leiomyosarcoma. The tumor was composed of cells of a single type, varying from ovoid to extremely elongated forms compactly arranged in a fasciculated pattern. Irregularly throughout the tumor were broad bands of collagenic tissue, in some parts forming septums. The centrally located nuclei seen when the neoplastic cells were cut longitudinally were ovoid, generally vesicular, with small but prominent nucleoli. An occasional mitotic figure was noted, also multinucleated forms (fig 3).

In addition to hematoxylin-eosin preparations, sections treated with Van Gieson's connective tissue and Masson's trichrome stains were studied. With Van Gieson's method the cytoplasm of the neoplastic cells appeared yellow, while with Masson's trichrome stain the cells were of a dusty brick red color.

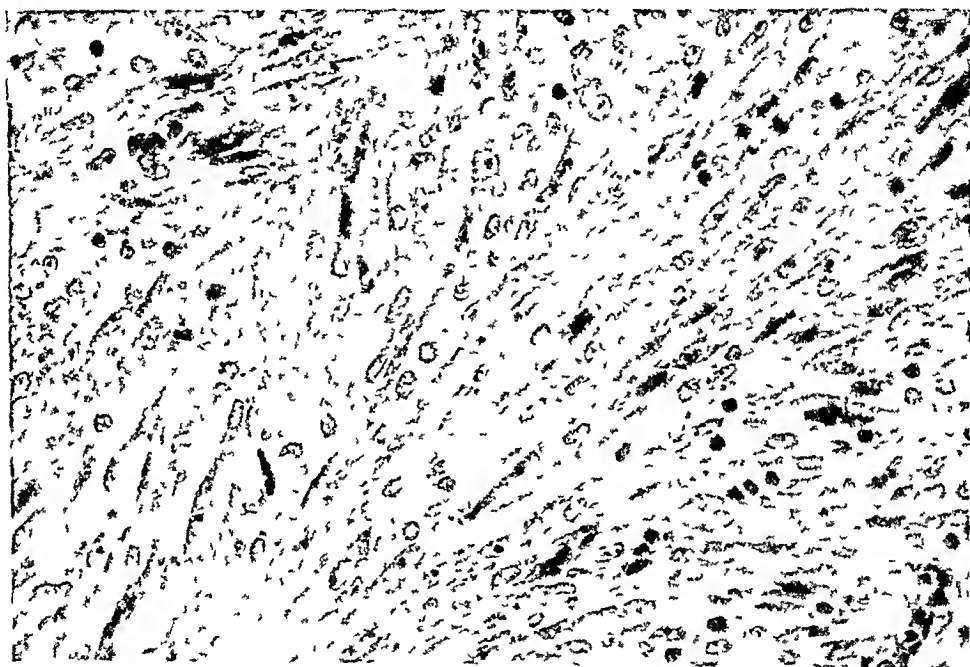


Fig 3—The morphologic aspects of the neoplastic cells are clearly shown in this photomicrograph. Note the variation from bluntly ovoid to extremely elongated forms with centrally located vesicular nuclei. Masson trichrome stain, $\times 400$.

The lung parenchyma surrounding the tumor was compressed, and in some areas neoplastic cells partially filled alveolar spaces. The neoplasm for the most part, however, was encapsulated by irregular but dense collagenic tissue.

COMMENT

A pulmonary lesion described by Rosendahl⁷ as diffuse myomatosis of the lungs is not to be confused with the solitary smooth muscle tumors, such as the one reported here.

Rosendahl's lesion and those described by von Stoessel,⁸ Berg and Vejens,⁹ and Berg and Zachrisson¹⁰ represent a myomatous transfor-

⁷ Rosendahl, T. *Acta radiol* 23 138, 1941.

mation of pulmonary tissue surrounding alveolar cavities, which for the greater part are highly distended. The genesis of this condition, if not neoplastic, has been attributed by von Stoessel, as a sequela, to chronic inflammation. This view, however, is not supported by the other authors mentioned. Necropsy in the cases reported by Berg and Vejens and Berg and Zachrisson revealed tuberous sclerosis of the brain, and these authors associated the smooth muscle lesions with the complex seen in tuberous sclerosis.

Rosendahl did not accept either of these views and left the question of genesis entirely open.

As for those lesions which are solitary and intimately associated with the larger bronchial structures, there is no reason to doubt their neoplastic nature. In the patient studied by us respiratory symptoms first developed three years prior to death, and the progression of the tumor was followed by repeated roentgen studies over a period of approximately one year. The symptoms and the physical findings were those of bronchial occlusion due to neoplasm and suggested, along with the roentgen findings, bronchiogenic carcinoma.

The location of the tumor, with its involvement of the cord, precluded surgical cure of the neoplasm. There was a reasonable chance, however, that extirpation of the lung and the tumor would prevent further hemorrhages and that even if a small amount of tumor tissue was left in the main bronchus the patient's life might be prolonged.

If the neoplasm had been proved to be a bronchiogenic carcinoma, a palliative operation would not have been recommended. In this case, however, tissue obtained through the bronchoscope demonstrated a low grade leiomyosarcoma. There was no possibility of favorable effects from roentgen therapy, and serious hemoptysis could not be controlled by any other means than surgical intervention.

SUMMARY

A 34 year old Negro man had a primary leiomyosarcoma of the right upper lobe bronchus. The tumor was diagnosed histologically before pneumonectomy was attempted. Six other cases of a mesenchymal neoplasm of bronchial origin of this type have been recorded in the literature.

W S R—Watts Hospital, Durham, N C

B B—1335 H Street, Washington, D C

8 von Stoessel, E. Beitr. z. Klin. d. Tuberk. **90** 432, 1937

9 Berg, G., and Vejens, G. Acta pædiat. **26** 16, 1939

10 Berg, G., and Zachrisson, C. G. Acta radiol. **22** 425, 1941

GLOMERULONEPHRITIS, WILMS'S TUMOR AND HORSESHOE KIDNEY IN AN INFANT

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THIS case is presented because of the unusual clinical picture and the interesting findings at autopsy

A 10 month old boy was referred to the University Hospitals because of a large mass in the abdomen. Since 3 months of age he had had many infections of the respiratory tract. In spite of these he had taken feedings well and developed normally. One month prior to his admission his mother noted that the left side of the abdomen was distended. There was concurrently a loss of appetite as well as irritability. Sulfadiazine was prescribed for a period of two days. One week prior to his admission, swelling about the face, the scrotum and the legs was noted, and the mother felt a large, firm mass in the abdomen. The child was taken to a urologist, who made the diagnosis of Wilms's tumor and recommended that the child be admitted to the hospital.

All other aspects of the history were entirely irrelative. The child represented the mother's second normal pregnancy, and the neonatal period had been free of complications.

Physical Examination—The patient's temperature was 98.6 F (rectum). The body weight was 9.12 Kg. The child appeared to be irritable but in no acute distress. The circumference of the head was 46 cm, and the fontanels were closed. Edema of the face was present, being most marked about the eyelids. The pupils were round and equal, reacting well to light. Slight convergent strabismus was present on the right side. The fundi were thought to show some generalized edema, most marked near the optic disk. The pharyngeal mucous membrane was slightly hyperemic. Some discrete small anterior cervical lymph nodes were palpable on the right side. The heart and the lungs were thought to be normal. The abdomen was distended but not noticeably tender. A large mass, which was firm, immovable and somewhat irregular, was palpable in the left side of the abdomen. It occupied the entire left portion of the abdominal cavity from the costal margin to the pelvis and from the midline to the lateral abdominal wall. Percussion revealed a slightly tympanic note over the right side of the abdomen, the left side gave a sound that was flat to dull. There were no changes in the findings with changes in position. Several small soft nodes were palpable in the inguinal regions bilaterally. Both testes were felt in the scrotum, which was noticeably edematous. The extremities appeared edematous, especially over the lower parts of both legs, where moderate pitting edema was found. Neurologic examination disclosed no abnormality.

Several examiners attempted to determine the blood pressure on repeated occasions. A small cuff was used, but there was failure to obliterate the radial pulse at a pressure of 300 mm of mercury.

From the Department of Pathology, University of Minnesota

The specific gravity of the urine was 1.023, the p_H value was 5. The urine contained albumin (3 plus), occasional leukocytes and finely granular casts were also present. A routine study of the blood revealed 112 Gm of hemoglobin per hundred cubic centimeters and 16,400 leukocytes per cubic millimeter, of which 52 per cent were neutrophils, 39 per cent lymphocytes, 5 per cent monocytes, 2 per cent eosinophils and 2 per cent basophils.



Fig 1—The tumor mass removed from a 10 month old boy who suffered from edema, hypoproteinemia, hypercholesteremia, albuminuria and severe hypertension.

Three days prior to the patient's admission roentgen studies had been done at the referring hospital to determine further the nature of the abdominal mass. A barium sulfate enema showed the colon displaced to the right by a large homogeneous soft tissue mass in the upper left region of the abdomen. As the splenic flexure was not depressed, the possibility of an enlarged spleen was

excluded. A gastrointestinal roentgen series showed the stomach and the small bowel displaced to the right.

Clinical Course—The patient was given a diet of purees suitable to a baby and routine pediatric care. He was prepared for intravenous urographic examination which was attempted on his second hospital day. This examination was unsatisfactory. A large mass was seen which apparently was in the posterior region of the abdomen, appearing to be identical with the kidney on the left side. A roentgenogram of the chest revealed a left ventricular type of cardiac enlargement.

The third day the child was noted to be more edematous and to have clinically demonstrable ascites. He had gained 0.43 Kg in weight since admission. The blood urea nitrogen was 50 mg, the blood cholesterol 676 mg and the plasma proteins 3.8 Gm per hundred cubic centimeters.

The patient continued to show increasing edema and ascites so that by the sixth hospital day his weight had increased 0.53 Kg more and by the eighth day 0.50 Kg more. Active treatment was limited to supportive measures, including a transfusion of 150 cc of whole blood on the sixth day, two days later 80 cc was given. The edema was progressive, and on the ninth hospital day an attempt was made to replace plasma proteins with concentrated plasma, 50 cc being given. Soon after this was administered, the patient became cyanotic and dyspneic. Numerous moist rales were heard over the entire chest. A diagnosis of cardiac decompensation was made and the infant placed in an oxygen tent. It was decided to digitalize the patient, and digalen, 0.25 cat unit (0.5 cc), was given intravenously. These measures failed to ameliorate the child's condition in any way, and the patient died on the evening of the ninth hospital day.

Autopsy—The right pleural cavity contained 75 cc, and the left 100 cc, of clear yellow fluid. The lungs showed hypostatic congestion and edema. The heart was enlarged, with moderate dilatation of the right atrium. The transverse diameter of the heart was 8.3 cm. The intrathoracic diameter of the chest was 15 cm. Hypertrophy of the left ventricular wall was observed, with the myocardium measuring 1.10 cm in thickness at the apex.

A large retroperitoneal mass occupied most of the abdominal cavity on the left, displacing the small intestine and the stomach to the right. It was found to originate from the upper pole of the left kidney and measured 14 by 5 by 6.5 cm (fig 1). It was grayish yellow and had a somewhat irregular surface. Microscopic sections were made from a number of different parts of the tumor. Over 90 per cent of the tissue was necrotic, but there were numerous islands of living tumor tissue. The necrotic tissue consisted largely of shadows of striated muscle fibers. The non-necrotic portions consisted chiefly of adult striated muscle fibers, but there were occasional areas of undifferentiated muscle, and a few tubules were found such as occur in adenosarcoma. The diagnosis was therefore Wilms's tumor (rhabdomyosarcoma).

The kidneys were united in the midline anterior to the vertebral bodies, presenting a typical horseshoe deformity. The left kidney measured 7 by 3 by 2.5 cm. and the right 8.5 by 3 by 2.7 cm. There was fetal lobulation. Several small cysts, measuring 0.2 to 1.0 cm in diameter, were visible. On section the cortex was found to be 8 mm in thickness. The cortical tissue was yellow, but the medulla showed no abnormality. The cysts were confined to a few small subcapsular areas in the cortex. The ureter and the bladder were normal.

The brain was examined grossly and sectioned. There were no demonstrable lesions.

Microscopically, the following changes were evident. Under low magnification it was noted that there was moderate diffuse tubular atrophy (fig 2). The glomeruli all showed endothelial proliferation of some degree with capillary obstruction, which was responsible for the tubular atrophy. Many of the glomeruli showed epithelial crescents, which were partly fibrous, as shown in figure 2. This is a characteristic picture of subacute glomerulonephritis.

COMMENT

The abdominal mass, edema, albuminuria, hypercholesteremia, severe hypertension and renal insufficiency constitute a most challenging clinical picture. The large retroperitoneal tumor apparently arising from the left kidney would make probable the clinical diagnosis of Wilms's tumor.



Fig 2—Photomicrograph of kidney

All the aspects of this case certainly could not be explained on the basis of this neoplasm alone.

It is now recognized that hypertension may occur in a child with Wilms's tumor. Bradley and Pincoff¹ have observed 5 consecutive patients with this tumor, all having arterial hypertension. In 2 patients removal of the tumor reduced the blood pressure to normal. Daniel² studied 18 patients, ranging in age from 8 months to 9 years, in all but 4 there was definite hypertension. These 4 patients were the only ones who were free of recurrence or metastasis at the time of the author's report. The highest pressure recorded in this series was 164 systolic.

1 Bradley, J. E., and Pincoff, M. C. *Ann Int Med* 2: 1613, 1938.

2 Daniel, W. E. *South M J* 32: 104, 1939.

and 130 diastolic Braasch, Walters and Hammer³ also studied 18 patients and found hypertension in only 5 Koons and Ruch⁴ removed a Wilms tumor from a 7 year old girl whose blood pressure was 180 systolic and 130 diastolic, and eleven days later the blood pressure had fallen to 116 systolic and 76 diastolic

It is evident therefore that Wilms's tumor can produce hypertension The mechanism involved in this is not known Daniel² expressed the belief that the relative renal ischemia is the causative factor Bradley and Pincoff¹ postulated that pressor substances secreted by the tumor tissue were responsible Their analysis of one Wilms tumor removed from a child with hypertension revealed that such substances were not present In no case reported has there been a degree of hypertension simulating that found in the case studied here It appears unlikely that Wilms's tumor alone could cause such extreme elevation of the blood pressure as was observed in this 10 month old infant One must conclude that other factors were present to explain not only this but other aspects of the case

The presence of glomerulonephritis in an infant under 1 year of age is not common⁵ Albuminuria, hypertension and uremia with absence of hematuria, may at times be found when this condition occurs during childhood The extensive glomerular damage observed in the infant studied can certainly be regarded as sufficient to explain the definite albuminuria and the uremia and may constitute the major factor in the production of the hypertension

Finding two relatively uncommon pathologic conditions in an organ also having congenital malformation, namely, a horseshoe kidney, is an extremely rare clinical event The horseshoe kidney has been reported observed at a rate varying from 1 in 385 autopsies (Shoemaker and Braasch) to 1 in 1,000 (Davidsohn) Eliason and Stevens⁶ have provided the one report in the literature of Wilms's tumor arising in a horseshoe kidney No report could be found, however, in which these two conditions were described to occur complicated by the presence of glomerulonephritis

The cardiac involvement in this case deserves brief mention, as it doubtless was the primary factor in the terminal episode Wessler⁵ in 1914 drew attention to the cardiac changes in nephritis in children He observed hypertrophy of the left ventricle with both acute and chronic glomerulonephritis and emphasized that such changes might develop in the relatively short period of a few weeks Left ventricular hypertrophy was found to be present in the case reported here to the extent that might be found in classic hypertensive heart disease in the adult Failure of the left side of the heart was the immediate cause of death The progress

3 Braasch, W F, Walters, W, and Hammer, H J J A M A **115** 1837, 1940

4 Koons, K M, and Ruch, M K J A M A **115** 1097, 1940

5 Wessler, H Arch Int Med **14** 517, 1914

6 Eliason, E L, and Stevens, I W Ann Surg **119** 788, 1944

of the terminal events would indicate that the failure of the heart was precipitated by the increase in the volume of blood following the administration of concentrated plasma. Rubin⁷ has reported two deaths in children with nephritis to whom a 50 per cent dextrose solution had been given intravenously as a diuretic.

SUMMARY

A 10 month old infant had progressive edema, an abdominal mass, severe hypertension, albuminuria, hypoproteinemia and definite hypercholesteremia. An autopsy revealed subacute glomerulonephritis, Wilms's tumor and horseshoe kidney.

⁷ Rubin, M. I., in Nelson, W. E. *Mitchell-Nelson Textbook of Pediatrics*, ed 4, Philadelphia, W. B. Saunders Company, 1945.

Books Received

RENAL DISEASES By E T Bell, M D, professor of pathology in the University of Minnesota, Minneapolis Pp 434, with 115 engravings and 4 color plates Price \$7 Philadelphia Lea & Febiger, 1946

This monograph is, according to the author's preface, "in part a compilation of studies on renal disease carried on by the author during the past twenty-five years" Much of the subject matter has been published in various journals, particularly in the *American Journal of Pathology* and the *ARCHIVES OF PATHOLOGY* But this new volume is far from being merely a compilation or a reprinting of old material It is a complete, well integrated presentation of diseases of the kidneys from the point of view of structural pathology, with a discussion of the correlated pathologic physiologic aspects and clinical manifestations

These studies are based on an analysis of all the cases of diseases of the kidneys mentioned in the records of 32,360 autopsies Since the subjects of these autopsies were derived from a large number of hospitals of all types—educational, charity and privately owned—and the number of cases of diseases of the kidneys is so large, deductions concerning the relative incidence of the various types of renal disease are statistically significant

The book is divided into twelve chapters The first chapter is a short classification of renal diseases The second presents the normal histology, and the third the normal and the pathologic physiology, of the kidneys These are followed by chapters on developmental anomalies, obstructions of the urinary tract—hydronephrosis, glomerulonephritis, tubular disease, acute interstitial nephritis (pyelonephritis), diseases of the blood vessels, diseases of the kidneys related to metabolic disturbances, extrarenal azotemia, tumors of the kidneys The volume is well and copiously illustrated with 115 charts, photographs of gross specimens and photomicrographs, including four colored plates The cases in each group and subgroup of diseases are tabulated in a manner that facilitates study and analysis, there are 73 tables Each subdivision is followed by an excellent bibliography pertaining to its particular subject At the end is an adequate index of 6 pages The author writes with admirable clearness, without decoration and without dullness

This monograph illustrates the modern concept of pathology and its place in medicine, namely, that the structural changes induced in an organ by disease furnish the soundest basis for an understanding of the functional disturbances that result from disease The entire volume will interest all pathologists Large sections of it will prove useful to practitioners of such diverse specialties as internal medicine and urology Even obstetricians will find much instruction in the section of 21 pages on the toxemias of pregnancy, based on a study of 52 cases that were examined at autopsy The work can be recommended as a comprehensive and illuminating discussion of all phases of the diseases that occur in that interesting and essential pair of organs, the kidneys

MONGOLISM AND CRETINISM A STUDY OF THE CLINICAL MANIFESTATIONS AND THE GENERAL PATHOLOGY OF PITUITARY AND THYROID DEFICIENCY By Clemens E Benda, M D, director, Wallace Research Laboratory for the Study of Mental Deficiency, Wrentham, Mass, and instructor in neuropathology, Harvard Medical School Price \$6.50 Pp 310, with 110 illustrations New York Grune and Stratton, 1946

The essential purpose of this book is to advance the knowledge and understanding of mongolism which is the most frequent disorder of growth of infancy It is estimated that there are 60,000 mongoloid persons in the United States and

that, of the 8,650 human beings born daily, 17 are probably mongoloid. The results of a study of mongolism as observed during ten years in some 300 persons of all ages from 2 days to 30 years, including 50 on whom autopsies were done, are analyzed and discussed. It is assumed that "in the mongoloid, nature has provided mankind with one of her strangest experiments—creating a human being without proper function of the pituitary," and the nature of the functional disturbance is not known. Features of cretinism are included in the presentation in order that this as yet poorly understood complicated disorder of growth may be compared with the disorder of growth caused by a single gland. Mongolism and cretinism are considered separately in the same chapters. After the historical introduction—mongolism was described first in 1866—come chapters on the physical characteristics and the diagnosis, the mental development, the nervous system, the morphologic changes in the endocrine glands, as well as those in other organs, the growth of the skeleton (especially that of the skull) and the results of roentgenologic, hematologic and biochemical examinations. The final chapters discuss the relation of the maternal condition to mongolism, the possibilities of its prevention, and its treatment. There is an author and subject index. Each chapter is provided with a comprehensive bibliography. There are 48 tabulations as well as numerous condensed clinical histories and descriptions of pituitary, thyroid and adrenal glands. The morphologic aspects on the whole are well illustrated, but one wishes that the pituitary gland of the mongoloid could have been more thoroughly studied and more clearly pictured. The book is an important contribution to the study of mongolism and should mark the beginning of comprehensively organized efforts to solve the problems of its causation and prevention.

LES TUMEURS ET LES POLYPES DU COEUR. ETUDE ANATOMO-CLINIQUE. By Dr Ivan Mahaim. Preface by Prof Dr Jean-Louis Nicod. Monograph of the Institute of Pathologic Anatomy of the University of Lausanne. Pp 568, with 67 illustrations. Paris: Masson & Cie [Lausanne: F Roth & Cie], 1945.

The author, a Swiss cardiologist of standing, is perhaps best known for his writings on the His-Tawara system. His present monograph is based on an exhaustive review of the literature and on a series of thorough personal observations. The first part of the book deals with the neoplastic and thrombotic polypoid formations of the heart, their anatomy, locations and effects, their diagnosis and the possibilities of surgical treatment. The details of 250 reported cases of auricular polypi are tabulated, the most frequent polypi are the myxomatous ones. The second and third parts concern the nonpolypoid benign tumors and the cancers of the heart, respectively. Cellothelioma or mesothelioma of the node of Tawara—Monckeberg's lymphangi endothelioma—receives special consideration, and a case observed by the author is described in detail and well illustrated. The main details of 87 instances of primary sarcoma of the heart are given in tabular form. Some 115 pages are devoted to the secondary tumors of the heart. Several cases studied with special reference to diagnosis by the author are recorded, and the bibliography of cardiac metastases is analyzed with respect to the origins of these. The fourth part discusses the tumors of the pericardium, and here again special stress is placed on diagnosis and treatment. The "general conclusions" review fundamental problems of diagnosis and the question of surgical treatment of cardiac tumors and polyps. A table lists 28 cases—2 original—in which a positive clinical diagnosis of cardiac tumor was made. A strong plea is made for great care in the etiologic diagnosis of cardiac insufficiency. The gross appearances of cardiac tumors are well illustrated. The bibliography lists 1,298 references. There is a table of the case reports in the text, and there are complete author and subject indexes. The book is a great storehouse of information about cardiac tumors and polyps and, as emphasized by Professor Nicod in its preface, it prepares the way for the expansion of cardiac surgery.

PATHOLOGIC CHANGES IN GOUTY ARTHRITIS

MARY S SHERMAN, M D
CHICAGO

IN SPITE of the relative frequency with which an accurate clinical diagnosis of gouty arthritis can be made,¹ there is seldom an opportunity to study the changes in bones and joints removed at operation, and there are few reports of such changes in the literature. In the present case, amputation performed for another condition afforded the opportunity to study several joints, and the pathologic observations will be reported in this paper.

REPORT OF A CASE

A 64 year old shipping clerk was admitted to the University of Chicago Clinics, Aug 2, 1945, because of inability to walk during the preceding four and a half months.

He had been told that when he was about 9 months old pain and swelling developed in his right knee. After these symptoms had subsided, he began to walk normally, but when he was 5 years old the knee "began to draw up." The deformity was treated by hamstring tenotomies, which resulted in a knee that was straight but stiff. Gradually the deformity returned, and for at least fifty years the knee had been fixed in 110 degree flexion. Every few months he would have a bout of pain in the knee which lasted a few days and subsided spontaneously. In spite of these difficulties, he had been able to work regularly.

Seven months before his admission he had an attack of dizziness without unconsciousness, after which he noted difficulty with his speech and weakness of his right arm and leg. Because of this he went to a hospital where he was treated by rest in bed for a "stroke." While he was there he was awakened one night by pain in his right great toe. The blood uric acid level the next day was 6.5 mg per hundred cubic centimeters, with all other laboratory tests normal, and the diagnosis of gout was made. Treatment with colchicum was instituted, the attack subsided, and the patient went home fairly well.

Two and a half months later he fell heavily on his right side, and from then on he had so much pain in his knee that he was unable to walk. If he sat still and made no attempt to bear weight he had no pain except at night after he was in bed.

The patient had also noted dyspnea and substernal pain on exertion. There was a history of tuberculosis in his father, brother and sister.

Physical examination revealed an obese elderly white man with little facial expression. His chest was normal. His blood pressure was 150 systolic and

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¹ McCracken, I P. Owen, P S, and Pratt J H. J A M A 131 367, 1946.



Fig 1—In *A* the narrowed cartilage spaces, the sclerosis of the subchondral bone and the irregular marginal lipping of the joints of the big toe are roentgenographic evidence of degenerative arthritis

B, marked degeneration and deformity of the joint surfaces of the right knee and atrophy of disuse of all bones. Note the concentric atrophy of the fibula, which suggests that the disability started in childhood

C, photograph of the metatarsophalangeal joint of the big toe showing deposits of urates. Note that there is no bony ankylosis

90 diastolic His right arm was weak and stiff, and the reflexes were hyperactive There was marked atrophy of the entire right lower extremity There was a 20 degree flexion deformity of the hip and moderate fixed external rotation The knee, in which there was barely perceptible motion, was flexed 110 degrees,

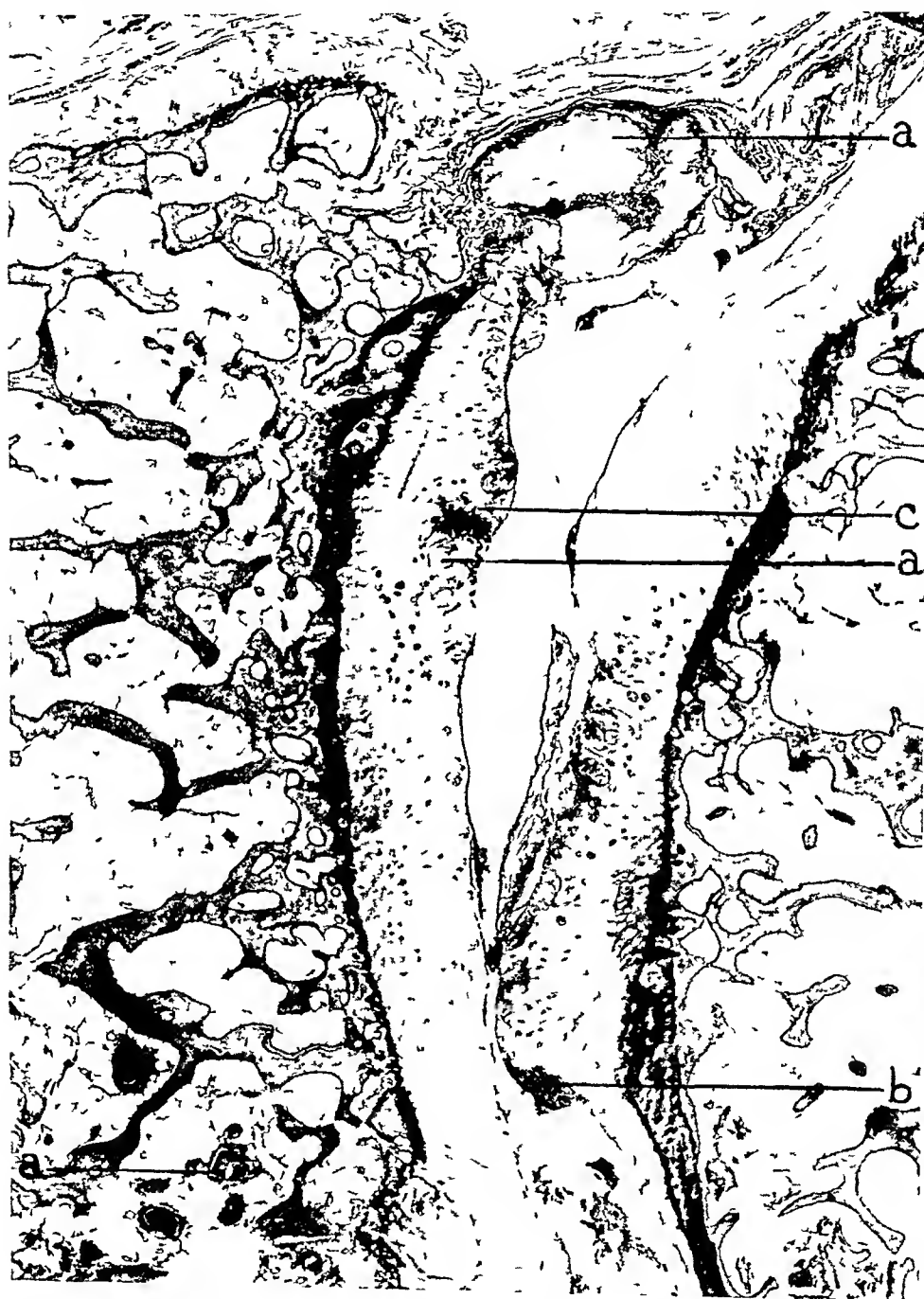


Fig 2—Photomicrograph of a sagittal section of the metatarsophalangeal joint showing urates deposited in the synovial membrane, the marrow, and the cartilage (a), a thick vascular synovial membrane growing over the surface as a pannus (b), a greatly degenerated articular cartilage, which in places is necrotic and beginning to calcify (c)

was swollen and felt warm. The right great toe was ankylosed at the metatarsophalangeal joint.

The results of laboratory examinations were all within normal limits except for the concentration of blood uric acid, which was 6.8 mg per hundred cubic centimeters. The roentgenogram of the chest was normal, and the electrocardiogram showed no pathognomonic changes. The roentgenogram of the foot showed moderate degenerative changes of the joints of the right great toe but nothing characteristic of gout (fig 1A). The roentgenogram of the knee showed definite evidence of degenerative changes and deformities compatible with old destructive arthritis (fig 1B). The cartilage space was narrowed, the subchondral cortex irregular, and there was advanced osteophyte formation.

Because of the patient's age and the fact that there was no possibility of restoring function to the knee joint, a low thigh amputation was performed. The patient had an uneventful postoperative course and two months later was walking with a prosthesis and crutches.

On the first and second postoperative days the blood uric acid levels were 9.18 and 9.40 mg per hundred cubic centimeters. Treatment was begun with colchicine, $\frac{1}{125}$ grain (0.5 mg) daily for two weeks. At this time medication was stopped because of anorexia, and one month later the uric acid level was 8.68 mg. Although the patient was without symptoms, his diet was adjusted, and five months later the blood uric acid level was 4.26 mg per hundred cubic centimeters.

On gross examination of the amputated limb, pathologic changes could be noted in all joints except the ankle. The knee joint was the most severely damaged. There was advanced destruction of the articular cartilages, and all surfaces were firmly bound together by abundant dense fibrous tissue. There was no bony ankylosis, but the shape of the articulating surfaces had become adapted to the position of extreme flexion so that even when they were freed the knee could not be extended. In a few spots, particularly on the femoral condyles, flaky white deposits were evident. When these were scraped off and examined under the microscope, they were seen to be composed of the needle-like crystals characteristic of the urates. A similar appearance was noted in the joints of the great toe which had been painful (fig 1C). The small joints of the foot, which had been symptom free, showed more advanced involvement. Where the deposits were scraped off there were revealed deep erosions of the articular cartilage. The synovial membrane in places was thickened and contained minute nodules.

A sagittal section through the motionless interphalangeal joint of the great toe revealed extensive changes (fig 2). Although a relatively thick layer of articular cartilage persisted, none of it was normal. It stained poorly, it was fibrillated, and the lacunae often contained large numbers of cells. Near the surface there were clumps of colorless amorphous material, around which cartilage cells were flattened, and about some of them, where there had been necrosis of the cartilage, calcification was present. On other portions of the surface erosion of the cartilage was being effected by a vascular type of fibrous pannus. This evidently arose from the synovial membrane, which was much thickened (fig 3A). The blood vessels were engorged, and there were many chronic inflammatory cells both in foci and scattered diffusely throughout. All through this tissue were many small and large deposits of the white amorphous material, about which lymphocytes and giant cells were gathered in a thick layer. At its juncture with the articular cartilage the proliferating synovial membrane was not only growing over the joint surface but also beneath the cartilage, which it was destroying from below.

The subchondral bone and fatty marrow were relatively normal except in a few places where there appeared small deposits of the amorphous material (fig 2). Under higher magnification these appeared to be situated in the marrow (fig 3*B*). The center of each deposit was more dense than the periphery, and



Fig 3—*A*, synovial membrane, much thickened, with many engorged blood vessels and nodular deposits of urates

B, deposit in marrow (see fig 2). Note the intense but limited inflammatory reaction and the great numbers of multinucleated foreign body giant cells

each one was surrounded by a narrow zone of intense inflammatory reaction. This shell of vascular connective tissue contained some round cells and so many large multinucleated foreign body giant cells that they formed almost a continuous layer.

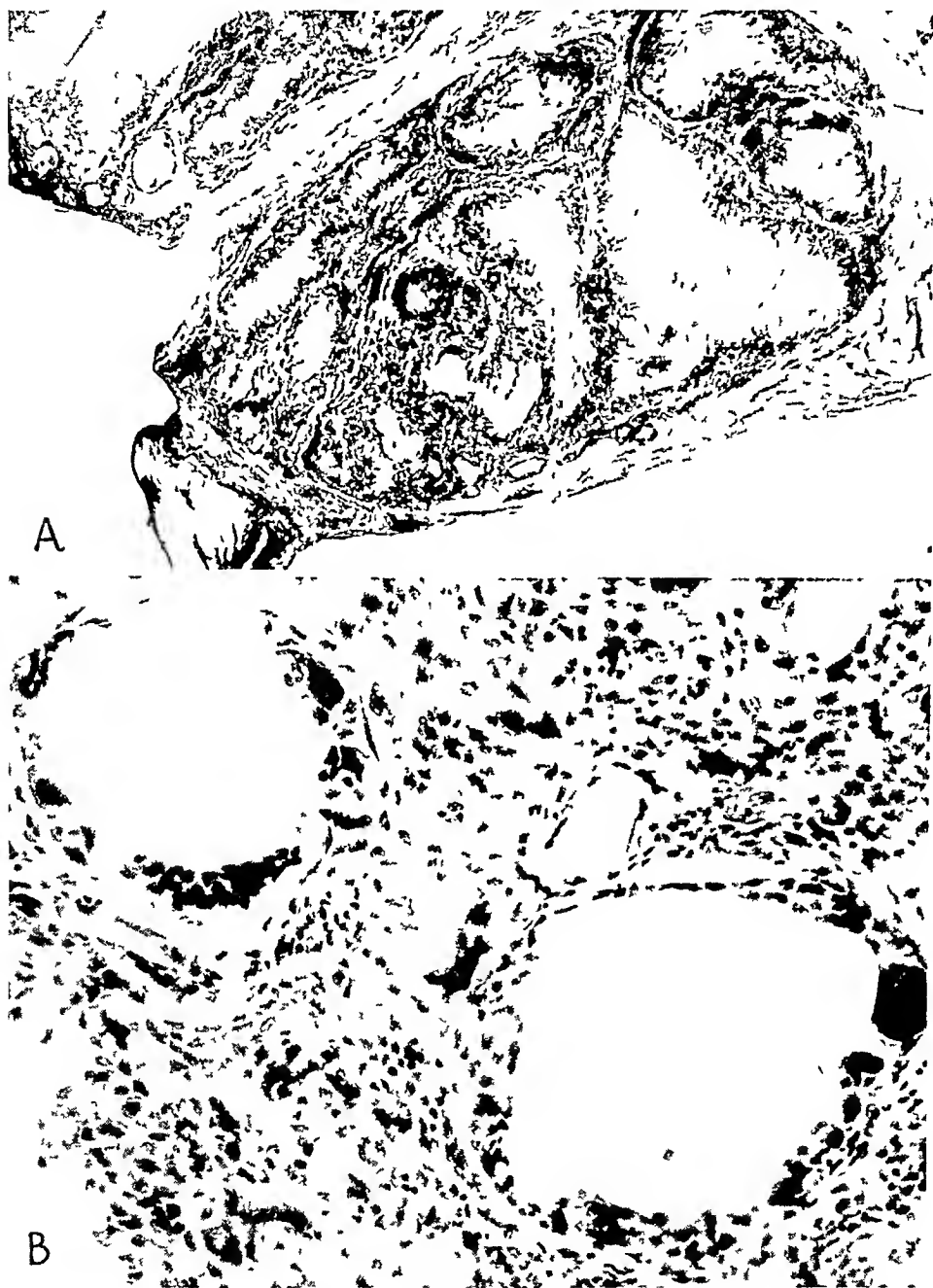


Fig 4—*A*, photomicrograph of material removed from a gouty tumor of an olecranon bursa of a patient with classic gout. Note the similarity to figure 3 *A*, with well demarcated nodules separated by fibrous connective tissue.

B, high power photomicrograph of the same lesion with rings of multinucleated giant cells surrounding nodules.

The assumption that the amorphous material surrounded by the foreign body reaction represented deposits of urates is supported by figure 4 *A* and *B*. These photomicrographs show changes identical with those illustrated in the preceding figures. The section was taken from a huge gouty deposit in an olecranon bursa of a patient who had classic gout. Smears of the fresh material showed urate crystals.

COMMENT

Routine fixation of material destroys the characteristic urate crystals of gouty deposits and leaves only the amorphous masses seen in the photomicrographs. For specific demonstration of the crystals in tissue, special technics must be employed.² However, the diagnosis can easily be confirmed by microscopic examination of a fragment of the deposit. Also, even after routine treatment, the pathologic changes are unmistakable.

Occasionally the deposit shows two definite concentric layers of crystals as noted in figure 3 *B* and less well in figure 4 *B*. Some workers have analyzed these nodules and found that the peripheral ring is usually composed of sodium urate and the central portion of cholesterol.³ This separation probably becomes more evident as the lesion grows older but even in the less well differentiated lesions cholesterol is usually present.⁴

The deposits are seen in articular cartilages, marrow, synovial membranes, joint capsules, bursae, ligaments and tendons.⁵ Whenever they occur they provoke an intense inflammatory reaction characterized by many large multinucleated foreign body giant cells which form a ring around the deposits. The synovial membrane of an involved joint thickens and proliferates to form a pannus on the articulating surfaces and also attacks the cartilage from below.⁶ In time there is a secondary response to the chronic irritation. The bony margins of the involved joints produce osteophytes, the subchondral bone becomes sclerotic, and the chronic changes of osteoarthritis are added to the acute changes caused by chemical irritation.⁷

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7 Moore, N. *St Barth Hosp Rep* **23** 289, 1887

BRAIN REPAIR

I Phospholipid-Splitting Enzymes of Brain Phagocytes

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THE MORPHOLOGIC changes following mechanical puncture of the brain have been studied in animals by a number of workers. Macklin and Macklin,¹ Russell² and Carmichael³ showed that the microglia cells, also known as macrophages and gutter cells, are the major participants in the acute reaction which follows mechanical puncture. These phagocytes, which are part of the reticuloendothelial system,⁴ slowly clean up the blood and the cellular detritus resulting from the injury. The number and the activity of these cells reach a maximum about the fifth day after injury. It has been shown quantitatively by Hicks and Opie⁵ that in the spleen these same phagocytes are capable of active proteolytic digestion of red and white cells, but their ability to digest myelin and other phospholipid-containing materials in the brain has not been demonstrated. It is well known that in the wound tracks of mechanical punctures of the brain phagocytes containing lipids and hemosiderin persist for periods of from weeks and months to almost a year after the initial injury. This phenomenon suggests that these cells are capable of only slow digestion of phospholipids derived from brain tissue and erythrocytes. Many tissues contain enzymes capable of splitting phosphoric acid from its combinations with organic substances.⁶ These enzymes have been loosely designated as "phosphatases." Normal brain tissue contains phosphatases which are able to produce slow autolysis. Giri and Datta⁷ have demonstrated in the brains of sheep phosphatases which split phosphoric acid from sodium beta glycerophosphate.

This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the United States Navy. The opinions and views set forth are those of the writer and are not to be considered as reflecting the policies of the Navy Department.

1 Macklin, C. C., and Macklin, M. T. *Arch Neurol & Psychiat* **3** 353, 1920

2 Russell, D. *Am J Path* **5** 451, 1929

3 Carmichael, E. A. *J Neurol & Psychopath* **9** 209, 1929

4 Dunning, H. S., and Furth, J. *Am J Path* **11** 895, 1935

5 Hicks, S. P., and Opie, E. L. *Am J Path* **18** 333, 1942

6 King, E. J. *Biochem J* **28** 476, 1924

7 Giri, K. V., and Datta, N. C. *Biochem J* **30** 1089, 1936

It is not feasible to isolate the phagocytes of the brain and measure their phosphatase activity. However, the tissue of a puncture wound rich in phagocytes can be excised and its phosphatase activity compared directly with that of similar normal brain tissue. Any difference between these two may be attributed to the enzyme activity of the phagocytes in the wound track. Another study, in which experimental punctures were made in the brains of 40 mice, which were then killed at varying intervals, showed that the number and the activity of the phagocytes had reached a maximum about five days after the injury, indicating that lesions of this age would present the best conditions for a study of the enzymes of the phagocytes.

Experiments were therefore undertaken in 30 mice to compare the phosphatase activity of wound track tissue five days old with that of normal brain tissue. This activity was studied in regard to both brain tissue substrate and sodium beta glycerophosphate substrate. Since it was necessary to excise some brain tissue along with the wound tracks, this could be measured and made to serve as brain substrate when desired. Sodium beta glycerophosphate, a convenient standard laboratory substrate, contains phosphorus linkages which are analogous to those in phospholipids. Therefore, in some experiments this substance was added to the already present brain substrate in order to magnify the effect of the phosphatase activity. The hydrogen ion concentration of p_H 7.2 at which the experiments were conducted was selected as being within the probable physiologic range of intracellular and extracellular fluids as found in the living brain. The significance of phosphatase activities of brain tissue carried on outside of the physiologic range of p_H 6.5 to 7.5 is doubtful for two reasons. (1) It is not known whether higher or lower concentrations of hydrogen ions occur in the living brain and (2) certain phosphatases are known to reverse their activities completely with large changes in hydrogen ion concentration, effecting cleavage of glycerophosphate at one concentration and synthesis at another.⁸

EXPERIMENTAL PROCEDURES AND RESULTS

Young adult male and female white mice between the ages of 6 weeks and 3 months were used in all experiments. Under ether anesthesia and aseptic conditions each cerebral hemisphere was punctured from the side with a 20 gage occluded hypodermic needle to a measured depth of 4 mm. The needle was introduced through the scalp and the skull and passed through the meninges and the cerebrum into the interbrain. The animals were killed rapidly with ether five days after injury, and the brains were removed and the enzyme studies started at once. All animals were submitted to autopsy, and none was remarkable except for the puncture wounds.

⁸ Gortner, R. A. *Outlines of Biochemistry*, ed. 2, New York, John Wiley & Sons, Inc., 1938, chap. 37, p. 942.

The sample of brain tissue in which phosphatase activity was to be measured was in each case ground fine in a mortar, suspended in barbital sodium buffer solution (2.12 Gm of monosodium diethylbarbiturate in 500 cc of distilled water). When it was desired to add glycerophosphate as a substrate to the already present brain tissue substrate, this was done in the amount of 2.5 Gm of sodium beta glycerophosphate ($\text{Na}_2 \text{C}_3\text{H}_5[\text{OH}]\cdot\text{PO}_4 \cdot 5\frac{1}{2}\text{H}_2\text{O}$) per 500 cc of barbital sodium buffer.⁹ The pH was adjusted to 7.2 in each case after the suspension was prepared by using 5 per cent hydrochloric acid without effective changes in volume. Bromthymol blue was used as the indicator. Incubation was carried out at 37 C in stoppered glass vessels, in the presence of toluene to prevent bacterial decomposition. Normal brain tissue for control studies was always taken from an area corresponding to the site of the wound track tissue, namely, the cerebrum. At the beginning of each experiment the initial phosphoric acid present in the suspension of brain tissue was determined as inorganic phosphorus by the colorimetric method of Kolmer and Boerner.⁹ This was computed and recorded as milligrams of inorganic phosphorus per gram of brain tissue. In all cases this was found to be from 0.5 to 0.9 mg, a range which is in good agreement with that recorded by Page¹⁰ and Randall.¹¹ At certain intervals during incubation samples were taken of each suspension and inorganic phosphorus determinations were made. These were also computed and recorded as milligrams of inorganic phosphorus per gram of brain but were corrected by subtracting from them the amount of inorganic phosphorus found in each case at the beginning of the experiments. Accordingly the recorded values of inorganic phosphorus in the tables represent the actual cumulative increase due to phosphatase activity incubation. Two series of experiments were conducted.

In the first series, the brains of 12 animals—4 normal controls and 8 whose brains had been punctured five days previously—were studied with regard to phosphatase activity toward both brain tissue substrate and glycerophosphate. In each case the entire forebrain was excised and trimmed to weigh exactly 300 mg, this was then made up to a 10 cc volume of suspension. This included the wound track if the brain had been punctured. Two of the normal brains and 4 of the punctured brains were thus each made up with plain barbital sodium buffer solution according to the method described. The two other normal brains and the 4 other punctured brains were each made up with barbital sodium buffer to which had been added sodium beta glycerophosphate substrate as described. The amount of inorganic phosphorus liberated by enzyme activity was expressed in milligrams per gram of brain tissue as previously noted. In that group of experiments in which brain tissue was the only substrate the total phosphorus available in 1 Gm of brain was approximately 3 mg, mostly in the form of phospholipid and therefore available for phosphatase activity.¹² In the group of experiments in which glycerophosphate was added, the calculated volume of suspension which would contain 1 Gm of brain tissue (33.3 cc) would have not only approximately

9 The barbital sodium buffer with glycerophosphate is that described for phosphatase and inorganic phosphorus determinations by J. Kolmer and F. Boerner (Approved Laboratory Technique, ed 2, New York, D. Appleton-Century Company, Inc., 1938, p. 756).

10 Page, I. H. Chemistry of the Brain, Springfield, Ill., Charles C. Thomas, Publisher, 1937, chap. 3, 10 and 11.

11 Randall, L. O. J. Biol. Chem. **124**: 481, 1938.

12 Page¹⁰ Randall¹¹.

3 mg of phosphorus in the form of phospholipid but also approximately 16 mg of phosphorus in the form of glycerophosphate. Therefore, if the enzyme action in each group of experiments went to completion, it would level off at about 3 mg per gram when only brain substrate was available and about 19 mg when both brain and glycerophosphate acted as substrates. In the experiment the enzyme reactions leveled off when only about half of the substrate was used up in each instance. The results are recorded in tables 1 and 2 and chart 1.

TABLE 1—*Phosphatase Activity Toward Brain Tissue Substrate by Normal Mouse Brains and Mouse Brains Containing Five Day Old Puncture Wound Tracks*

| | Increase in Milligrams of Inorganic Phosphorus Liberated by 1 Gm of Brain Tissue During 72 Hr at p_H 7.2 and 37 C * | | |
|-------------------------------|---|-------|-------|
| | 24 Hr | 48 Hr | 72 Hr |
| Normal brain | 0.8 | 1.6 | 1.5 |
| Normal brain | 0.9 | 1.6 | 1.6 |
| Brain with 5 day wound tracks | 0.6 | 1.6 | 1.4 |
| Brain with 5 day wound tracks | 0.7 | 1.5 | 1.6 |
| Brain with 5 day wound tracks | 0.6 | 1.3 | 1.2 |
| Brain with 5 day wound tracks | 0.6 | 1.5 | 1.6 |

* Total available phosphorus 3 mg per gram of brain tissue

If a smooth curve be drawn through the successive values of inorganic phosphorus in each experiment in tables 1 and 2, the difference between normal and punctured brain is not greater than the difference within the normal group or within the punctured group. Therefore there is no significant difference, in these experiments, between the phosphatase activity of normal brain and that of punctured brain.

TABLE 2—*Phosphatase Activity Toward Brain Tissue Substrate with Added Sodium Beta Glycerophosphate by Normal Mouse Brains and Mouse Brains Containing Five Day Old Puncture Wound Tracks*

| | Increase in Milligrams of Inorganic Phosphorus Liberated by 1 Gm of Brain Tissue During 72 Hr at p_H 7.2 and 37 C * | | | |
|-------------------------------|---|-------|-------|-------|
| | 6 Hr | 24 Hr | 48 Hr | 72 Hr |
| Normal brain | 1.7 | 4.1 | 7.9 | 8.1 |
| Normal brain | 1.9 | 4.6 | 8.4 | 8.6 |
| Brain with 5 day wound tracks | 1.9 | 4.7 | 8.7 | 10.2 |
| Brain with 5 day wound tracks | 1.9 | 5.0 | 9.5 | 10.1 |
| Brain with 5 day wound tracks | 2.0 | 4.9 | 9.1 | 10.8 |
| Brain with 5 day wound tracks | 2.2 | 5.1 | 9.2 | 9.8 |

* Total available phosphorus 19 mg per gram of brain tissue

In order to determine the effect of a change in hydrogen ion concentration within the physiologic range, the concentration in each case shown in tables 1 and 2 was changed from p_H 7.2 to p_H 6.5 at the end of seventy-two hours, and incubation at 37 C was carried out for an additional twenty-four hours. There was no increase in liberated inorganic phosphorus, indicating that this change of hydrogen ion concentration did not effect a change in phosphatase activity.

Since no significant difference in phosphatase activity between the normal and the injured tissue was revealed in the first series of experiments tabulated in tables 1 and 2 and chart 1, a second series was carried out in which a relatively more concentrated suspension of wound track tissue was used. This was done because the first experiments involved considerable dilution of the tissue of the actual wound tracks. Small differences in the enzyme activity between normal and abnormal tissue might have been obscured by this dilution. In this second series of experiments the brains of 18 animals were used, i. e., 5 normal control brains, 11 brains with wound tracks five days old, and 2 brains which had been punctured just before the animals were killed for the study. These two fresh wound track controls were included in order to rule out the possible phosphatase activity of blood, for even after five days there is still some blood in the wound track. From each brain only 50 mg of tissue including the wound track in the cases of puncture

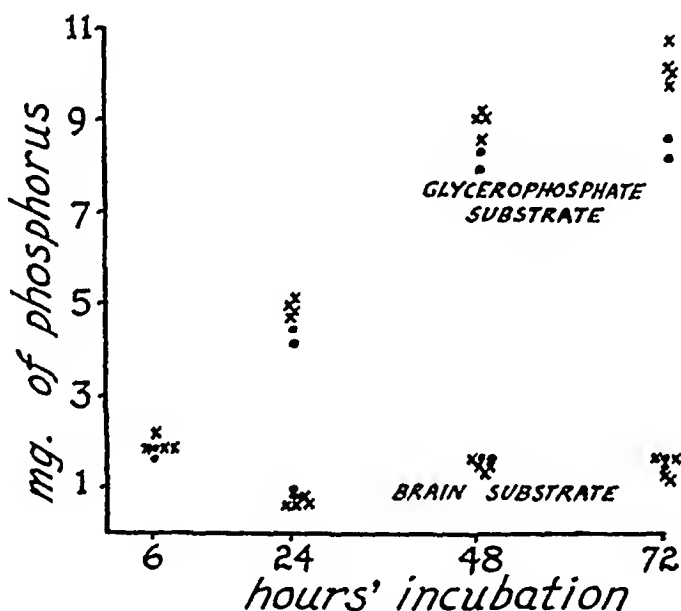


Chart 1—Graphic presentation of phosphorus determinations on control and experimental mouse brains of the first series of experiments (see tables 1 and 2). Normal brains are represented by x, punctured brains by •.

of the brain, was excised and made up to only 1 cc of suspension. The technique was in all other respects identical with that described for the first series of experiments. Three of the normal brain controls, 1 control brain with fresh wound tracks and 6 of the brains with five day old wound tracks were studied for phosphatase activity toward brain substrate only. This brain substrate was provided, as before, by the brain tissue excised. A similar group of the 8 other brains was studied for activities toward brain substrate with sodium beta glycerophosphate added in the manner previously described. The results are recorded in tables 3 and 4. In the group in which brain tissue was the only substrate, the total available phosphorus computed for the volume of suspension containing 1 Gm of brain was, as before, approximately 3 mg. However, when the glycerophosphate was added, the total available phosphorus in a volume of suspension (20 cc) which would contain 1 Gm of brain tissue was

3 mg from the brain and 10 mg from the glycerophosphate Van Slyke¹³ has pointed out that in hydrolytic enzyme reactions where there is an excess of substrate as in these experiments the reaction velocity is best observed in the first part of the reaction curve and is proportional to the enzyme activity. Therefore, it was necessary only to measure the inorganic phosphorus liberated in this second series during the first twenty-four hours, for in the first series of experiments the curve of maximum phosphatase activity was shown to be established during the first twenty-four hours. The results are recorded in tables 3 and 4 and chart 2.

TABLE 3—*Phosphatase Activity Toward Brain Tissue Substrate by Normal Mouse Brains and Mouse Brains Containing Five Day Old Punctate Wound Tracks*

| | Milligrams of Inorganic Phosphorus Liberated by 1 Gm. of Brain Tissue During 24 Hr pH 7.2 and 37 C * |
|-------------------------------|---|
| Normal brain | 0.7 |
| Normal brain | 0.5 |
| Normal brain | 0.8 |
| Brain with fresh wound tracks | 0.8 |
| Brain with 5 day wound tracks | 0.8 |
| Brain with 5 day wound tracks | 0.9 |
| Brain with 5 day wound tracks | 0.9 |
| Brain with 5 day wound tracks | 0.9 |
| Brain with 5 day wound tracks | 0.9 |
| Brain with 5 day wound tracks | 0.8 |

* Total available phosphorus 3 mg per gram of brain tissue

TABLE 4—*Phosphatase Activity Toward Brain Tissue Substrate with Added Sodium Beta Glycerophosphate by Normal Mouse Brains and Mouse Brains Containing Five Day Old Punctate Wound Tracks*

| | Milligrams of Inorganic Phosphorus Liberated by 1 Gm. of Brain Tissue During 24 Hr pH 7.2 and 37 C * |
|-------------------------------|---|
| Normal brain | 4.2 |
| Normal brain | 4.2 |
| Brain with fresh wound tracks | 3.6 |
| Brain with 5 day wound tracks | 4.6 |
| Brain with 5 day wound tracks | 4.4 |
| Brain with 5 day wound tracks | 5.0 |
| Brain with 5 day wound tracks | 5.4 |
| Brain with 5 day wound tracks | 4.8 |

* Total available phosphorus 13 mg per gram of brain tissue

Again as in the first series of experiments there is no significant difference in phosphatase activity between normal brain and brain containing a five day old wound track, despite the relatively high concentration of wound track tissue rich in phagocytes in this series.

COMMENT

The results of the experiments demonstrated that the tissue from a five day old wound track in the mouse brain did not possess a signifi-

¹³ Van Slyke, D. D., in Nord, F. F., and Werkman, C. H. *Advances in Enzymology*, New York, Interscience Publishers, Inc., 1942, vol. 2, p. 33.

cantly greater phosphatase activity than corresponding normal brain. At this time, five days after injury, histologic studies showed that the wound tracks contained a maximum number of phagocytes at peak activity. The inference may be made, then, that the phagocytes do not possess a greater quantity of phosphatases capable of splitting phosphoric acid from myelin, dead brain tissue, erythrocyte phospholipids and sodium beta glycerophosphate than does normal brain tissue. The technical necessity of using the whole wound track tissue, since a method for isolating the phagocytes remains to be accomplished, limits the accuracy of the experiments to the extent that very small differences in enzyme activity cannot be detected. Nevertheless these same cells

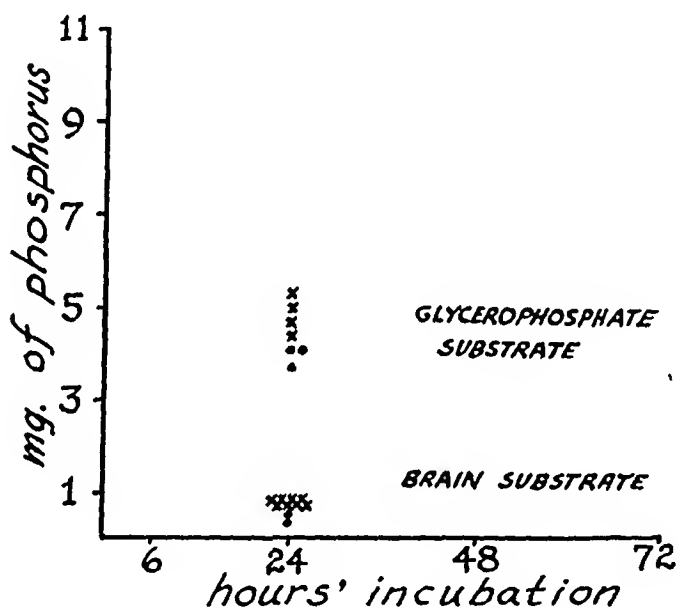


Chart 2—Graphic presentation of phosphorus determinations on control and experimental mouse brains of the second series of experiments (see tables 3 and 4). Normal brains are represented by x, punctured brains by •.

when active in the spleen, as noted in a foregoing paragraph,⁵ have been shown under similar experimental circumstances to possess marked proteolytic enzymatic activity when phagocytosing red and white blood corpuscles. In such experiments the difference in enzymatic activity between normal spleen and spleen containing large numbers of phagocytes was marked. Thus it would seem that these cells are well equipped to dispose of proteins, but with phospholipids their ability is at best mediocre.

It may be hoped that these experiments will lead to further studies of the enzyme activities of the cells concerned with the response to injury in various tissues. More refined tests for the assay of the

enzymes of the cells of the reticuloendothelial system may be perfected. Such studies are important for the understanding of the fundamental reactions in tissue injury.

SUMMARY

The phosphatase activities of normal mouse brains and mouse brains containing punctured wound tracks five days old and rich in phagocytes, were compared. Both brain tissue (phospholipids) and sodium beta glycerophosphate were employed as substrates.

No significant difference between the phosphatase activity of normal brain and that of wound track tissue was demonstrated.

It was concluded that these brain phagocytes, members of the so-called reticuloendothelial system, are not capable of splitting phospholipids to any noticeable degree. This is found to be in contrast to the proteolytic enzymatic capabilities of this same type of cell.

This sluggish enzymatic activity toward breakdown products of injured brain tissue is in agreement with histologic studies of the brain which show phagocytes containing lipid at sites of injury often persisting for many months.

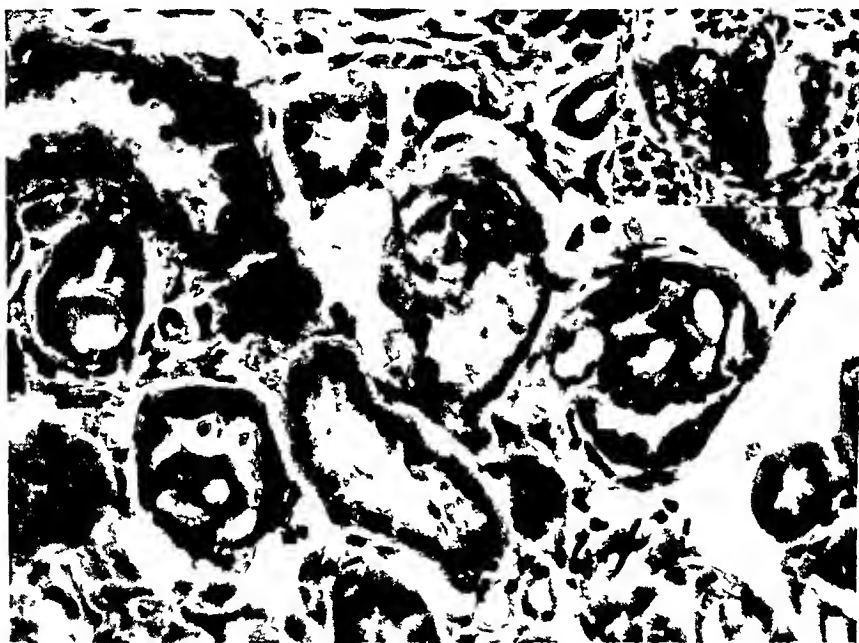
RENAL INTRATUBULAR SYNCYTIAL MASSES

A Note on Their Origin

ELIZABETH LOWENHAUPT, M D

SAN FRANCISCO

A MULTINUCLEATED cell response in the renal tubule related apparently to abnormal intraluminal contents, has been extensively described and discussed in cases of multiple myeloma¹ and noted in cases of poisoning by mercuric chloride - In regard to the former condition it has been suggested² that the giant cells are formed by the fusion of macrophages that enter the tubule from without In a previous



Kidney of rat after forty-two days of dietary chloride deficiency Four tubules are lined by regenerating epithelium, which forms multinucleated cells In three these cells surround vacuoles, sites of absorbed calcific material (Bouin's fluid fixation) Hematoxylin and eosin $\times 420$ The inset shows a similar reaction in acetone-fixed tissue, calcific material being present Gomori's alkaline phosphatase stain, $\times 115$

From the Department of Pathology, University of California

- 1 Forbus, W D , Perlzweig, W A , Parfentjev, I A , and Burwell, J C Jr
Bull Johns Hopkins Hosp **57** 47, 1935
- 2 Harmon, E L Am J Path **4** 321, 1928
- 3 Bell, E T Am J Path **9** 393, 1933

study ⁴ cells of similar appearance were noted in the kidneys of rats on a chloride-deficient diet and there too appeared related to precipitate within the tubular lumen. Since little comment is found in regard to other possibilities as to the origin of these cell sheets, it seems of interest to illustrate and briefly discuss the lesion.

Material and methods have been described previously,⁴ and the figure serves to show the lesion under discussion, which was noted uniformly in the material examined. Four tubules in the illustration are filled with cell masses, which appear to be formed by proliferating cells rather than by desquamation of sheets of degenerating epithelium. This is indicated by the fact that subsidiary tubules are present within each mass and that each of these smaller tubules is lined by flattened cells suggestive of regenerated tubular epithelium. In addition, cell structures remain well preserved. Likewise, it would appear that these cells are related to foreign material present within the lumen, in contact with them, and perhaps are proliferating in response to this precipitate—indicated by the inclusion of calcific granules within the cell cytoplasm. These points are shown in the figure.

SUMMARY

The observations described suggest that multinucleated structures in renal tubules may arise directly from the tubular epithelial cells, proliferating perhaps in response to contact with abnormal material—in this case calcium.

⁴ Lowenhaupt, E, and Greenberg, D. M. *Arch. Path.* **42**: 35, 1946.

MYOCARDIAL GRANULOMAS IN SUBACUTE BACTERIAL ENDOCARDITIS

OTTO SAPHIR, M D
CHICAGO

IN INSTANCES of subacute bacterial endocarditis, various inflammatory changes have been observed in the myocardium¹ These changes may be diffuse but are more frequently localized Commonly, perivascular round cell infiltrations are noted, but true abscesses are rarely encountered Often, also, small emboli are seen within branches of the coronary arteries, and minute infarcts, either recent or in various stages of organization If numerous sections are cut from the myocardium, these changes can easily be demonstrated

In addition to these diffuse or localized inflammatory or vascular changes, granulomas are occasionally encountered Foremost among them is the Aschoff body In a previous study,² in children, Aschoff bodies were encountered in the myocardium in 14 of 35 hearts with subacute bacterial endocarditis Libman and Friedberg³ recently stated that Aschoff bodies are present in about 25 to 45 per cent of cases of this disease On the other hand, Gelfman⁴ reported Aschoff bodies in only 2 of 50 instances of subacute bacterial endocarditis This discrepancy may be explained by the fact that not all investigators examine a comparable number of blocks from such hearts These lesions are significant because they constitute the only findings which indicate that the primary lesion of the heart valve was rheumatic in origin

Aschoff bodies may be present in the various but still recognizable stages Among 55 instances of subacute bacterial endocarditis, outspoken Aschoff bodies were found in 19 They were more commonly encountered in children and adolescents (under 20 years of age) Among 15 of the latter group they were found in 11 This may be explained by the possibility that relatively more of the myocardium is examined

From the Department of Pathology, Michael Reese Hospital

This study was aided by a grant from the A B Kuppenheimer Fund This department is supported in part by the Michael Reese Research Foundation

1 Saphir, O Am J Path **11** 143, 1935

2 Saphir, O, and Wile, S A Am Heart J **9** 29, 1933

3 Libman, E, and Friedberg, C K Subacute Bacterial Endocarditis, New York, Oxford University Press, 1941

4 Gelfman, R Ann Int Med **19** 253, 1943

histologically in the smaller hearts than in the adult heart if the number of sections examined in both age groups is the same. Aschoff bodies occurring in hearts which are the seat of subacute bacterial endocarditis have been the subject of much discussion. Since a review of such a discussion would lead too far afield, mention may be made only of the recent view of Kelson and White,⁵ who stated that two types of relationships appear to be present. Subacute bacterial endocarditis may act as a specific or a nonspecific factor to activate rheumatic fever in susceptible subjects, or subacute bacterial endocarditis may occur during the course of rheumatic fever.

Bracht-Wachter bodies are frequently mentioned as being characteristic of subacute bacterial endocarditis. Perry⁶ thought these embolic in nature. In a series of 9 consecutive cases such cellular foci were found in the myocardium in all but 1, and in this case the vegetations were confined to the tricuspid valve. He emphasized that in the earlier stages the cells appear to be polymorphonuclear leukocytes and lymphocytes in almost equal numbers, later the polymorphonuclear leukocytes become fewer and an occasional endothelial cell is seen. Libman and Friedberg³ stressed that Bracht-Wachter bodies replace the muscles and are not lesions of interstitial tissue. They spoke of localized collections of lymphocytes and mononuclear cells. White⁷ stated that Bracht-Wachter bodies had been reported as myocardial lesions more or less typical of subacute bacterial endocarditis. He described them as areas of mononuclear cell infiltration of the interstitial tissue of the myocardium. He further remarked that they are found sometimes in other cardiac infections and that they are not as specific for subacute bacterial endocarditis as are the Aschoff bodies for rheumatic heart infection.

This short review indicates the confusion which prevails in regard to the Bracht-Wachter body. The variation in the descriptions of this structure can easily be explained by the fact that Bracht and Wachter did not describe a single entity. Because of the discrepancies as to just what constitutes a Bracht-Wachter body, it may be of interest to review Bracht and Wachter's original publication in more detail.

Bracht and Wachter⁸ studied the hearts of patients who died as a result of acute infectious disease and also those of 4 patients for whom the clinical diagnosis of acute rheumatic arthritis had been made. The purpose of their investigation was to see whether or not Aschoff bodies are found exclusively in rheumatic hearts. They reported the

5 Kelson, S. R., and White, P. D. *Ann Int Med* 22: 40, 1945.

6 Perry, C. B. *Bacterial Endocarditis*, Bristol, John Wright & Sons, Ltd., 1936.

7 White, P. D. *Heart Disease*, ed. 3, New York, The Macmillan Company, 1944.

8 Bracht, E., and Wachter. *Deutsches Arch f klin Med* 96: 493, 1909.

finding of Aschoff bodies in 3 of 4 hearts of patients with acute rheumatic fever but in none of the hearts taken from patients who had died of other infectious diseases. Bacteriologic cultures of the blood taken from 2 of the 3 hearts in which Aschoff bodies had been found disclosed diplostreptococci resembling *Streptococcus viridans*. To see whether or not these streptococci would produce Aschoff bodies, they injected intravenously the streptococci found in each of these cases into 2 rabbits. Into a third rabbit they injected streptococci which were isolated from the heart blood of one of the original 2 rabbits.

Rabbit 1 was given five intravenous injections within forty-eight hours and was killed on the ninth day. Microscopically, minute and small areas of necrosis and cloudy swelling were found in the myocardium. Larger areas of necrosis were surrounded by lymphocytes and fibroblasts, and among the latter were occasional giant cells. Rabbit 2 was given four injections over a period of fourteen days and died spontaneously. There were three minute verrucae at the line of closure of the mitral valve and one on the tricuspid valve. The myocardium disclosed circumscribed areas of cellular infiltrations within the interstitial tissue, occasionally involving the heart muscle fibers. There were also foci of necrosis of muscle fibers and accumulations of lymphocytes, fibroblasts and isolated plasma cells. Rabbit 3 was given four injections over a period of sixteen days and then killed. Verrucae were found on the mitral and the tricuspid valve. Scar tissue was found replacing muscle fibers, and also some calcification was encountered.

From the foregoing review it is clear that only 3 rabbits were used. In 1 rabbits, however, myocardial changes of the type described may occur spontaneously.

For control experiments Biacht and Wachter used 3 rabbits. Two of the rabbits were given intravenous injections of streptococci obtained from a paronychia and 1 rabbit was given intravenous injections of streptococci isolated from infected tonsils. Microscopically, circumscribed areas with central necrosis infiltrated by polymorphonuclear leukocytes were found in the myocardium of these rabbits (abscesses).

From a critical review of the study published by Biacht and Wachter it is clear that the authors worked with no material obtained in cases of subacute bacterial endocarditis. They worked with only a few animals of a species in which myocardial changes occur spontaneously. Their "series" of experiments was carried out on 3 test rabbits and 3 control rabbits. Their findings were definitely not uniform. In view of this it is difficult to comprehend why in the literature the term "Biacht-Wachter bodies" was ever applied to myocardial lesions in subacute bacterial endocarditis. This is particularly confusing since these authors nowhere mentioned myocardial changes in subacute bacterial endocarditis.

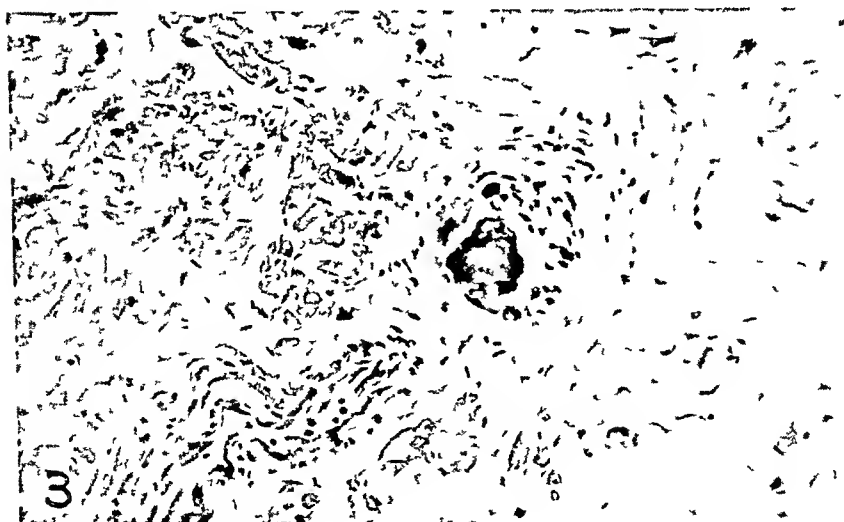
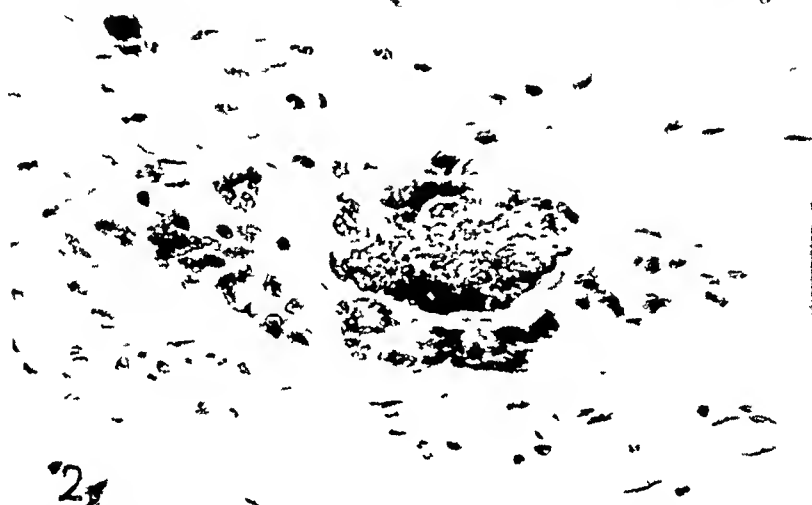
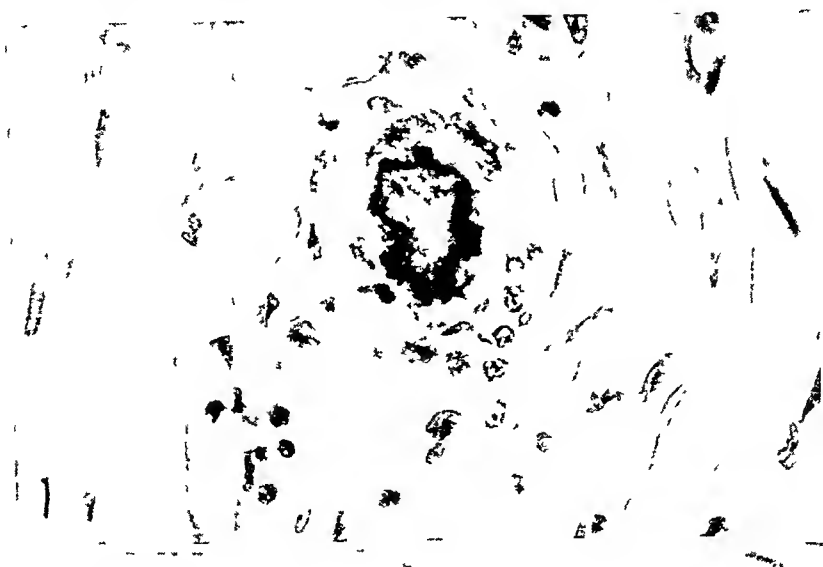


Fig 1—Dark amorphous material surrounded by foreign body giant cells Hematoxylin-eosin preparation, $\times 580$

Fig 2—Calcific material surrounded by giant cells situated in the interstitial tissue of the myocardium Van Gieson preparation, $\times 380$

Fig 3—Foreign body granuloma with the calcium deposit in the center Hematoxylin-eosin preparation, $\times 150$

The majority of communications which discuss Bracht-Wachter bodies and which have appeared since the original study was published show structures which correspond to those illustrated by figure 6 of the original report. These are designated as Bracht-Wachter bodies. Interestingly enough, figure 6 depicts what the original authors named "experimentally produced myocarditis in rabbits following injections of streptococci taken from paronychia." It demonstrates principally areas infiltrated by polymorphonuclear leukocytes.

It is thus clear that Bracht-Wachter bodies in the sense of granulomas specific for, or even characteristic of, subacute bacterial endocarditis do not exist, and that what have been described as Bracht-Wachter bodies are a variety of nonspecific lesions which perhaps may be produced experimentally by intravenous injections of streptococci. What Bracht and Wachter have described has nothing to do with subacute bacterial endocarditis. In a previous communication it has been stressed that the term "Bracht-Wachter bodies" should be discarded.¹ DeVasquez⁹ also has favored dropping the term. A study of 15 additional cases of subacute bacterial endocarditis further discredits the use of the term "Bracht-Wachter bodies."

In recently observed instances of subacute bacterial endocarditis, granulomas of a different variety have been encountered in the myocardium. Within the centers of these a small amount of a dark blue-stained (hematoxylin and eosin preparation) amorphous material was found, which stained black with silver (von Kossa). This material, obviously containing calcium, was surrounded by giant cells of the foreign body type and endothelial leukocytes. Only few lymphocytes were seen at the farther periphery. These granulomas, though principally present in the myocardium, were also found in the epicardium adjacent to the myocardium. Occasionally they were surrounded only by endothelial leukocytes and a few lymphocytes. In seemingly early instances this bluish amorphous material could be seen within capillaries or arterioles. A search for bacteria within this amorphous material gave negative results. From this description it is clear that these granulomas were foreign body granulomas, each surrounding a calcific particle.

It is of interest that these granulomas have not been disclosed by previous studies of the myocardium in cases of subacute bacterial endocarditis. They were found in 4 recent instances. The 4 patients had died during the last three years and had been treated extensively with various sulfonamide compounds and with penicillin. Subacute bacterial endocarditis of the mitral and aortic valves was found in all patients at autopsy. Evidence of healing in the form of organizing and organized vegetations of the aortic valve and the formation of

peculiar healing and healed erosive mycotic aneurysms of the mitral valve (to be the subject of a future study) were encountered. It may be merely mentioned that examination of the vegetations of the aortic

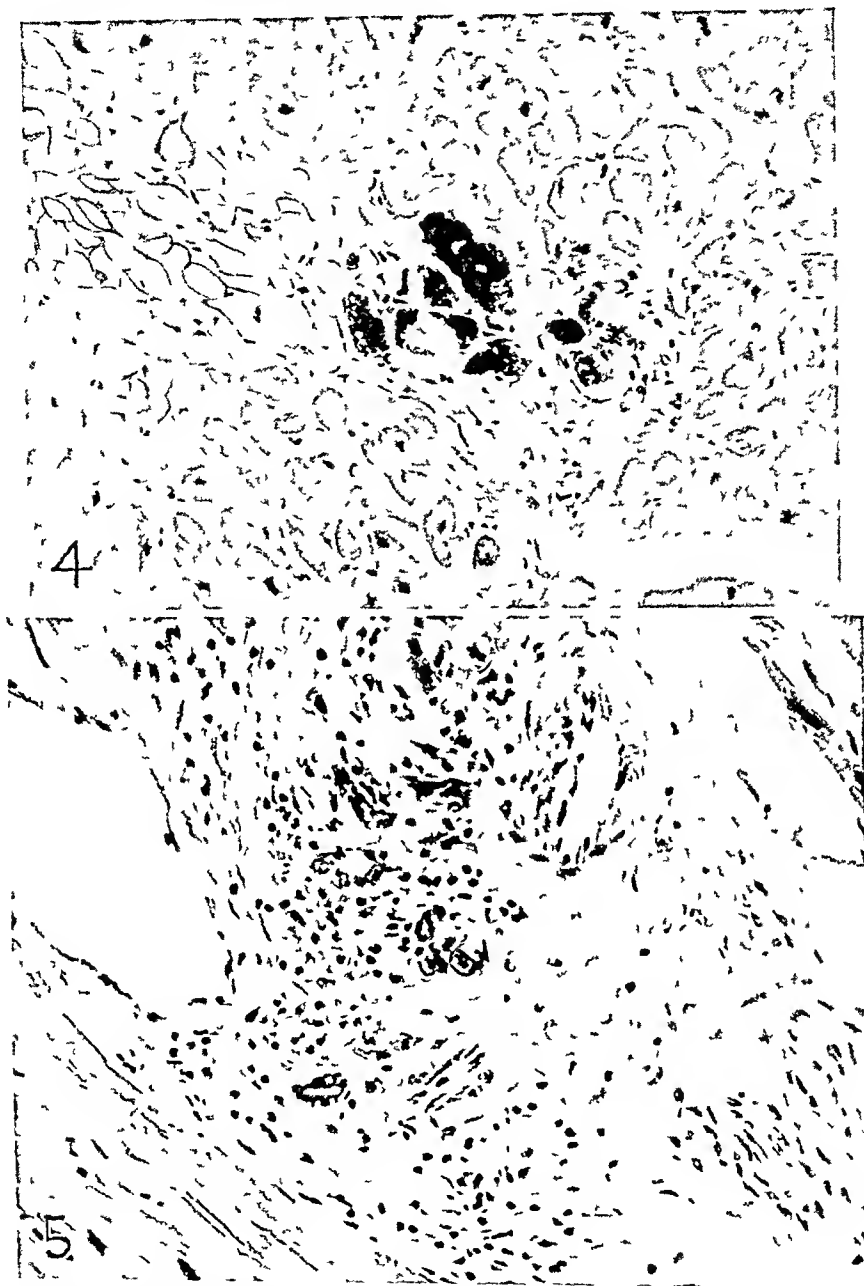


Fig 4—Dark amorphous material, some of which is situated in the interstitial tissue. Hematoxylin-eosin preparation, $\times 250$.

Fig 5—Calcific material, a few foreign body giant cells and lymphocytic infiltrations situated in the interstitial tissue. Iron-hematoxylin-eosin preparations, $\times 300$.

valve disclosed the presence of granulation tissue at their base and the absence of bacteria. At the periphery of the vegetations patches of

necrosis with few bacteria were noted in addition to smaller or larger amounts of a bluish amorphous material which histochemically was calcium. Only an insignificant amount of granulation tissue was observed in such areas. Thus the outstanding findings were organization at the base of the vegetations, and necrosis and calcific changes at the free margin.

Because of the fact that precipitated calcium is present within those portions of the vegetations which still show necrosis and little granulation tissue, particles can break loose easily and cause the formation of emboli. Morphologically it seems that necrosis, few bacteria and the simultaneous precipitation of lime salts are characteristic of instances in which therapeutic agents have altered the pathologic appearance of subacute bacterial endocarditis.

Emboli are encountered frequently in branches of the coronary arteries in subacute bacterial endocarditis. As a matter of fact, it had been noted previously that minute infarcts of the myocardium in various stages of healing were the most frequent and perhaps the most commonly encountered single change. The emboli so far described were clumps of bacteria and fibrin or particles from vegetations. None of these emboli calls for the formation of a foreign body granuloma. However, in those instances in which the embolus consists of lime salts, a foreign body reaction in the form of a foreign body granuloma ensues. It might be mentioned that in one of these 4 instances a similar foreign body granuloma was encountered in the kidney.

SUMMARY

A study of the myocardium in instances of subacute bacterial endocarditis disclosed, in addition to diffuse inflammatory changes, two types of granulomatous lesions. One of these, the Aschoff nodule, was found in 19 of 55 instances of subacute bacterial endocarditis. It was present in the myocardium of 11 of the 15 children studied. The other is a foreign body granuloma, obviously caused by calcific deposits, the result of calcific emboli arising from healing vegetations of the aortic valve. These were found only in recently observed instances of subacute bacterial endocarditis, in patients who had been treated with sulfonamide compounds or penicillin.

A critical consideration of the literature on Bracht-Wächter bodies indicates that they signify no definite entity and that, therefore, the term "Bracht-Wächter body" should be discarded.

ORGANIZED EMBOLI OF THE TERTIARY PULMONARY ARTERIES

An Unusual Cause of Cor Pulmonale

BENJAMIN CASTLEMAN, M D

AND

EDWARD F BLAND, M D

BOSTON

CHRONIC COR PULMONALE is the result of long-standing strain of the right ventricle secondary to circulatory obstruction in the lung. The usual causes are emphysema, fibrosis and silicosis, but occasionally primary alterations of the intima or the media of the pulmonary arteries are responsible. A considerable number of cases falling in the latter etiologic group have been studied in this hospital during the past twenty years, and their clinical course is to be reported in the near future. However, one of these is unique from a pathologic standpoint and warrants a separate report.

D C, a housewife aged 44, was under observation in the Massachusetts General hospital and in the outpatient clinic at frequent intervals during the nine years of her slowly progressive and ultimately fatal illness. In childhood she had measles, mumps, whooping cough and chickenpox. At the age of 29, appendectomy and salpingo-oophorectomy on the right side incident to a tubal pregnancy were done at another hospital. There were no postoperative complications. At the ages of 31, 33 and 35, respectively, she had normal pregnancies and delivered her offsprings at home, without showing cardiovascular symptoms.

Shortly after the last pregnancy, cyanosis of the lips was noted. This sign persisted with slowly increasing intensity throughout the remaining eight years of the patient's life.

At the age of 36 she began to have intermittent sharp pain in the epigastrium and lower substernal region, lasting from a few minutes to several hours, usually associated with a dry cough, and after a few months dyspnea on effort became evident and she entered the hospital. The significant findings were orthopnea, slight cyanosis of the lips, no clubbing, normal blood pressure, no cardiac murmurs but an abnormally loud pulmonary second sound, right axis deviation and large P waves by electrocardiogram (fig 1), and enlargement of the right ventricle and of the pulmonary conus by roentgenogram, without enlargement of the auricles.

From the Department of Pathology and Bacteriology and the Cardiac Laboratory, Massachusetts General Hospital

Circulatory studies supplied the following data

| | | Normal |
|--|---------------------------|--------|
| Circulation time | | |
| Saccharin, arm to tongue | 51.6 seconds | 20 |
| Ether, arm to lungs | 20.6 seconds | 10 |
| Crude pulmonary | 11.0 seconds | |
| Venous pressure (arm) | 125 mm of saline solution | |
| Red cell count | 6,000,000 | |
| Hemoglobin | 113 per cent | |
| Arterial blood (determinations by John H. Talbott) | | |
| Alveolar carbon dioxide-combining power | 22.0 mm of mercury | 40 |
| Arterial carbon dioxide-combining power | 22.4 mm of mercury | 40 |
| Oxygen capacity | 26.1 volume per cent | 20 |
| Saturation | 93.1 per cent | 95 |
| Cell volume | 57.9 per cent | 42 |
| Total carbon dioxide | 43.4 volume per cent | 58 |
| pH | 7.54 | 7.40 |

It is of interest that the circulation rates suggested obstruction proximal to the lungs, whereas the blood gas studies slightly favored congenital heart disease.

The subsequent seven years were characterized by slowly increasing failure of the right side of the heart, manifested by progressive cardiac enlargement, per-

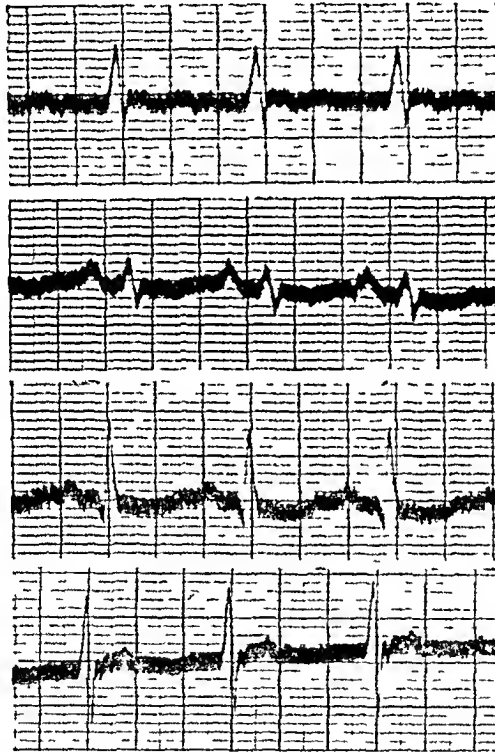


Fig 1—Electrocardiogram (leads I, II, III and IV) showing right axis deviation and low T waves

sistent gallop rhythm, venous distention, swelling of the liver and peripheral edema, requiring digitalis and diuretic therapy and occasional hospitalizations for more intensive treatment.

During the final year of the patient's life she required mercuraphylline injection, U S P, twice a week to control the failure of the right side of the heart. The lungs remained clear throughout the illness (fig 2). A painful umbilical hernia developed and added considerably to the patient's discomfort. Ultimately, operation with the region under local anesthesia was performed, but the wound

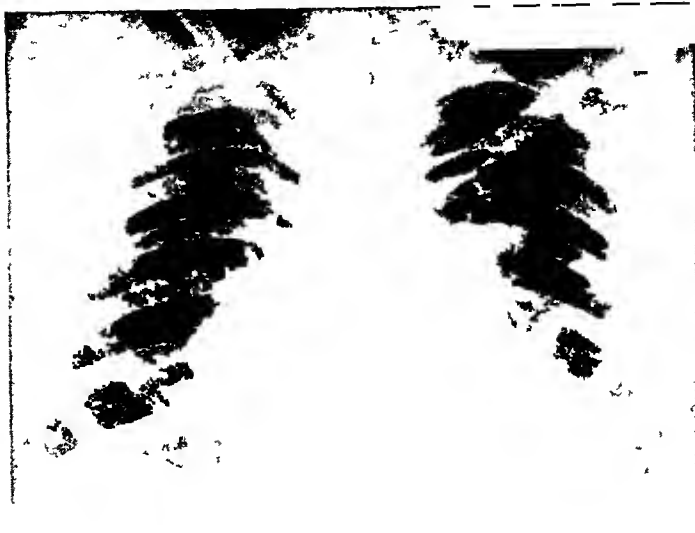


Fig 2—Roentgenogram made twelve days before death, showing marked enlargement of the heart The heart measures 18.3 cm, the thorax, 30.0 cm

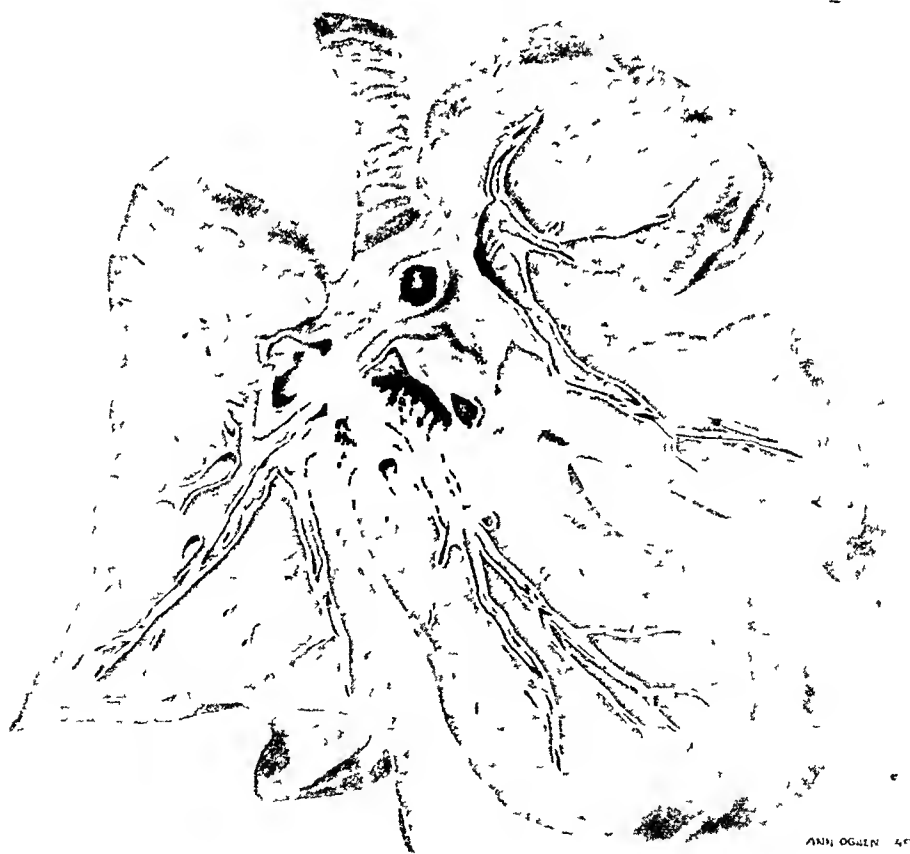


Fig 3—Drawing of a section of the right lung showing lacelike occlusions of tertiary branches of the pulmonary arteries Note the thickness of the wall proximal to the obstruction

disrupted on the third day, and she weakened progressively and died on the thirteenth day, nine years after the onset of her initial cyanosis. The terminal illness was complicated by ileus, mild jaundice and persistent failure of the right side of the heart.

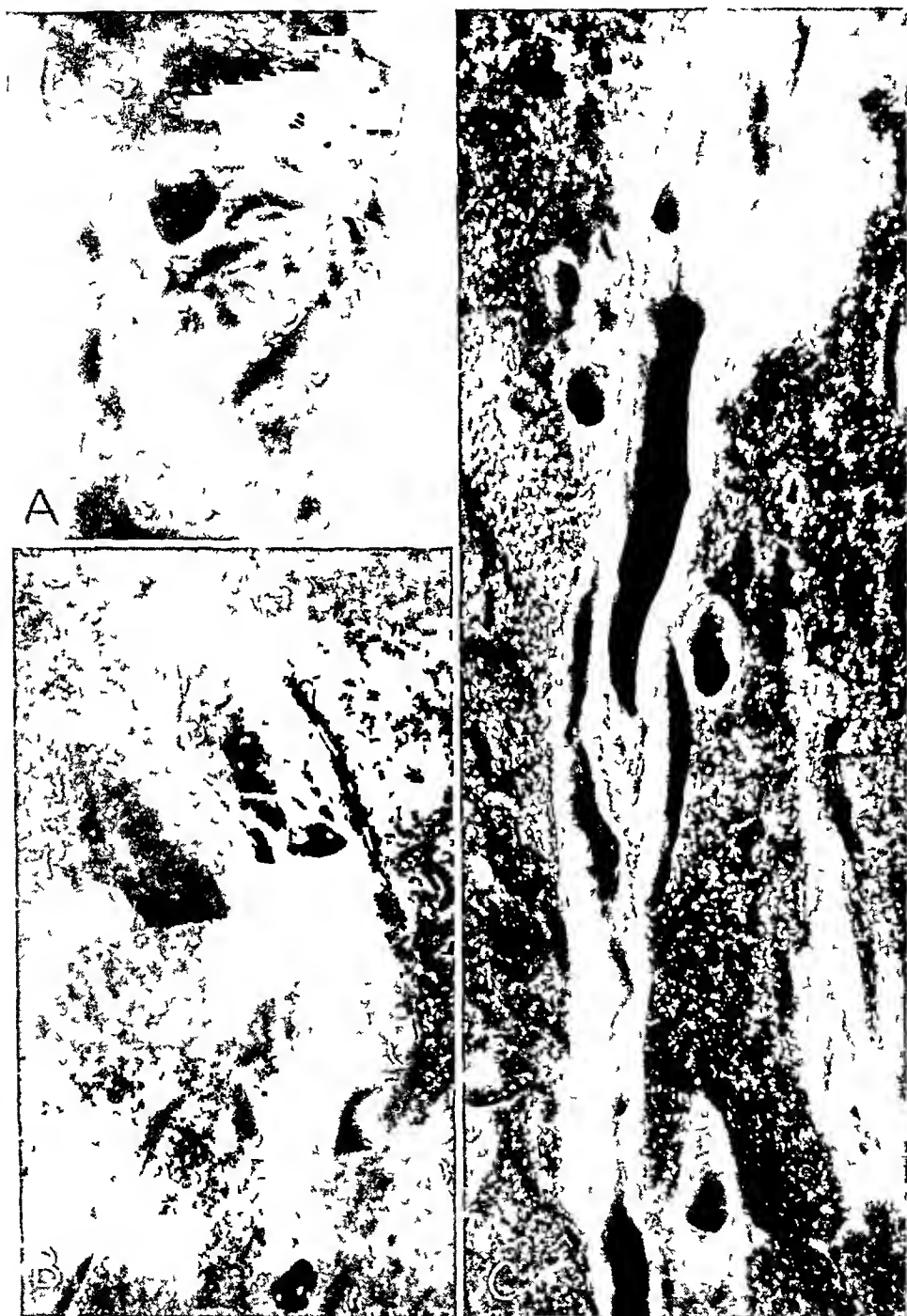


Fig 4—*A* and *B*, cross sections through points of obstruction, showing the latticework appearance of the fibrous trabeculae. *C*, photograph of one of the pulmonary arteries shown in figure 3. The atheromatous thickening proximal to the obstruction is conspicuous in contrast to the thin wall distal to the occlusion.

The postmortem examination showed that the immediate cause of death was acute general peritonitis

The heart was enlarged, weighing 520 Gm. There were marked dilatation and hypertrophy of the right auricle and ventricle, the latter measuring from 6 to 10 mm in thickness. The left ventricle was small, and its wall measured only 10 to 12 mm. The valves were normal. The coronary arteries and the aorta showed only slight atheromatous changes.

The unusual findings were in the pulmonary arteries. Practically every tertiary branch was occluded between 2 and 4 cm from its origin by a meshwork of thin tough fibrous trabeculae 5 to 10 mm in length (fig 3). On cross section they had a latticework appearance, but water could not pass the obstruction even under considerable pressure (fig 4 *A* and *B*). In the interstices of some of these occluding lesions was clotted blood, most of which was clotted post mortem, but some



Fig 5—One of main pulmonary arteries showing a valvelike pocket of the intima (arrow). Note also the cross section of one of the occluded tertiary branches (double arrows).

was clotted definitely ante mortem and organized. The main pulmonary artery, its primary and secondary branches, and those portions of the tertiary branches proximal to the obstructions were thick, and the intima was covered with atheromatous plaques. In sharp contrast, the vessel walls beyond the lesion were thin, atrophic and completely free from atheroma (fig 4 *C*). This difference was so striking that the exact point of obstruction in each vessel could be predicted on sectioning the lung, as long as the vessel wall was thick and atheromatous, the obstruction was still distal to the section level. In one of the main pulmonary arteries there was a small valvelike semilunar pocket of the intima, measuring 7 to 8 mm in diameter, with its opening away from the pulmonary valve (fig 5). This lesion was reminiscent of the so-called accessory aortic cusp occasionally seen below the aortic valve in long-standing aortic regurgitation.



Figure 6
(See legend on opposite page)

Microscopic examination of these meshworks showed that they were composed of fibrous tissue in which occurred numerous vascular spaces, varying in size and lined with endothelium. Some of these channels were filled with fresh blood, others, with organized thrombi, still others were empty. Elastic tissue preparations revealed that not only was the wall of the main vessel composed of dense elastic fibers, but a large amount of elastica was present throughout the walls of the small vascular channels within the lumen. Many of these apparently intraluminal vessels showed extensive fibrous intimal proliferation. The entire picture of these lesions is consistent with organized and recanalized thrombi.

The vessels proximal to the obstructions showed extensive fibrous intimal proliferation, but their lumens were not diminished to any appreciable extent. The obstruction in the tertiary branches was not a gradual narrowing but a strikingly abrupt process. Beyond the obstruction the vessels that grossly appeared thin and atrophic in contrast to the proximal thick portion were actually thicker than normal when viewed microscopically, and a few showed slight fibrous intimal proliferation. Some of the smaller arteries in and just beneath the pleura showed marked medial hypertrophy.

Another interesting finding was the presence of several pleural fibrous plaques, 1 to 3 cm in diameter, which extended into the parenchyma for distances of 3 to 10 mm. Some of these were puckered and dimpled on the surface. Grossly and microscopically they presented the characteristic appearance of the healed infarcts previously described by one of us (B C).¹

The liver weighed 1,800 Gm and showed well advanced cardiac cirrhosis.

COMMENT

When this patient was first seen in the Massachusetts General Hospital eight years before she died, she already had well marked clinical and laboratory evidence of cor pulmonale. Thus we can assume that the initiating pathologic cause probably occurred at least some years before that time.

One interpretation of the findings in the pulmonary arteries is to consider them as advanced arteriosclerosis of a primary nature. In fact, Rosenthal,² in a report of 3 cases of sclerosis of the pulmonary artery, in one of which (case 3) the arterial changes were not unlike those in our case microscopically, did believe that his case was merely an instance of severe arteriosclerosis. If this were true of our case, it would be difficult to explain why this overgrowth of nodular vascular tissue should occur only in the tertiary branches at points relatively equidistant from the hilus.

1 Castleman, B. Arch Path **30** 130, 1940

2 Rosenthal, S R. Arch Path **10** 717, 1930

EXPLANATION OF FIGURE 6

Photomicrographs (elastic tissue preparations) of cross sections through obstructions, showing the extensive organization and recanalization. Note the absence of sclerosis or of extension of the thrombus into a branch in A.

The almost constant location of the obstructing lesions suggested that perhaps they were the result of some embryologic maldevelopment, such as a rete mirabile, but Dr J Lewis Bremer, emeritus professor of anatomy at the Harvard Medical School, studied the material and was unable to account for these lesions on a developmental basis

This leaves two further possibilities—organized and recanalized thrombi or emboli. If thrombi, they would almost certainly have been formed on the basis of arteriosclerosis, but why always in the tertiary branches? One would have to assume that the patient had idiopathic sclerosis of the pulmonary arteries long before the development of the obstructing lesions. This is a definite possibility, namely, that a long-standing sclerosis slowly narrowed the lumen to such an extent that thrombi would be prone to develop and more so in the smaller tertiary branches

A more plausible explanation is that the lesions were the result of the lodging of small emboli in the tertiary branches, which then were organized and recanalized. The recanalization, however, was not sufficient to allow much, if any, blood to flow through most of the lesions. Since the same-sized small pulmonary arteries were involved, one would have to assume that the emboli came from thrombi in small vessels, the most likely source being the pelvic veins or possibly those of the lower part of the legs. Unfortunately, these veins were not investigated at autopsy by the prosector. It seems more likely that emboli would lodge in approximately the same-sized vessel than that thrombi would develop on the basis of the previous arteriosclerosis always in a tertiary branch and never in a primary or a secondary one. It is unlikely that these emboli were produced by amniotic fluid with epithelial squamæ as has been reported by Steiner and Lushbaugh,³ because in those cases the capillaries and very small arteries were occluded and not the main tertiary branches

We should like to suggest, therefore, that some years before the patient first entered the hospital, perhaps after the pelvic operation or one of the pregnancies, thrombi developed in the pelvic veins, which began to shower the lungs with emboli. Some of these emboli went far enough out in the lung to produce infarcts, since healed infarcts were found at autopsy, but most of them were sufficiently proximal to the pleura to allow collateral circulation and thus prevent infarction. The pulmonary pressure proximal to the emboli was thus increased and led to the severe atherosclerosis in contrast to the relative absence of atheroma beyond the obstruction. The dilatation and slight atheroma of the vessels beyond the obstruction can be accounted for by the increased pressure in the collateral circulation, part of which must have come

3 Steiner, P E, and Lushbaugh, C C. *J A M A* **117** 1245 and 1340, 1941

from the bronchial arteries. Evidence for the latter's role in the collateral circulation is the marked thickening of the pleural and subpleural arteries which are derived from the bronchial arteries.

SUMMARY

A 44 year old woman presented clinical evidence of cor pulmonale and slowly progressive heart failure for nine years. Postmortem examination showed localized occlusion of almost every tertiary pulmonary artery by what were believed to be organized and recanalized emboli. Evidence of marked hypertension in the pulmonary circuit was the severe atherosclerosis that ended abruptly in each vessel at the point of obstruction.

ABSENCE OF THE LEFT CARDIAC VENTRICLE WITH APLASIA OF THE AORTIC ORIFICE AND HYPOPLASIA OF THE AORTA

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TOTAL absence of the left ventricle in man is so rare a condition that no instance is included in the list of 1,000 cases of cardiac anomalies in Abbott's Atlas¹. The only comparable instance is one mentioned by Krumbhaar². This unreported case was observed at the University of Pennsylvania Hospital by Dr. W. F. Sheldon (no. 41-1249). As in my case, the heart had no trace of the left ventricle or of the mitral or the aortic valve. Unlike the heart in my case, it had a large foramen ovale. Walls³ reported a case in which one ventricle gave rise to a well developed aorta as well as to a normal pulmonary artery. In this, as in other instances of biatrial, trilobular hearts, there is a common or undivided ventricle rather than a solitary right or a solitary left ventricle. While simple arrest of development is an adequate explanation for the persistence of a common or undivided ventricle, there is no satisfactory explanation for true absence of the left ventricle. "Detorsion defects" such as were discussed by Shapiro⁴ do not seem adequate⁵. Intrauterine endocarditis is a possible explanation, particularly in the presence of demonstrable inflammatory changes⁶. In my case, however, there was no trace of inflammation or of scarring.

REPORT OF A CASE

A full term white girl was born at the Bayonne Hospital by low forceps extraction on June 26, 1944, at 4:35 a. m. Because she was cyanotic, she was given a breathing mixture of oxygen and carbon dioxide. Respirations remained labored, and at 8:10 a. m., three hours and thirty-five minutes after birth, she died. The mother, 28 years old, had had a normal child previously. The progress of her labor in the present case had been entirely normal. The fetal heart rate before

From the Department of Pathology, Bayonne Hospital and Dispensary, Bayonne, N. J.

1 Abbott, M. E. Atlas of Congenital Cardiac Disease, New York, American Heart Association, 1936.

2 Krumbhaar, E. B. J. Mt. Sinai Hosp. 8:737, 1942.

3 Walls, E. W. Lancet 2:668, 1941.

4 Shapiro, P. F. Arch. Path. 9:54, 1930.

5 Bremer, J. L. Arch. Path. 34:1016, 1942.

6 von Zalka, E. Frankfurt Ztschr. f. Path. 30:144, 1924.

birth had been 130 per minute. Owing to the short period of postnatal observation, no clinical diagnosis was made.

At necropsy, eight hours after death, the child weighed 8 pounds, 3 ounces (3,714 Gm) and was well developed and well nourished. The skin was pale. There were a few drops of clear fluid in the pericardial cavity. The pericardial surfaces were smooth and shiny. The heart measured 4.5 cm from base to apex and 4 cm across the base and weighed approximately 22 Gm. The apex was blunt and was made up of the right ventricle. The right atrium was about 2 cm wide, the endocardium was smooth and shiny. The superior and inferior venae cavae and the coronary sinus entered in the usual way. There was no trace of a foramen ovale, nor was there any other atrial septal defect. The wall of the right atrium varied between 0.1 and 0.2 cm in thickness, the wall of the auricle was thinner. The right atrioventricular orifice was 5 cm in circumference, the three leaflets were delicate.

The right ventricle formed the bulk of the heart. Its wall varied between 0.3 and 0.7 cm in thickness, including the trabeculae carneae. The papillary muscles were slightly thickened. The pulmonic orifice was 2.8 cm in circumference, and the three leaflets were delicate. Two of them appeared to be anterior and one posterior. The orifices of the right and left pulmonary arteries were in the postero-lateral aspects of the main vessel and were located, respectively, 0.2 cm and 0.7 cm above the pulmonic orifice. The intimal surface of the pulmonary artery was smooth and shiny. Where the widely patent ductus arteriosus opened into the aorta, its intimal surface was wrinkled and finely pitted and its wall was slightly thickened. The pulmonary artery continued as the ductus arteriosus into the descending aorta, the level of transition being marked by the arch of the hypoplastic aorta opening into the descending aorta. The entire segment from the level of this communication down to the level of the left branch of the pulmonary artery is to be considered the ductus arteriosus. This is shown in figure 9 of Popjak's⁷ report of a case of atresia of the aorta and hypoplasia of the left ventricle.

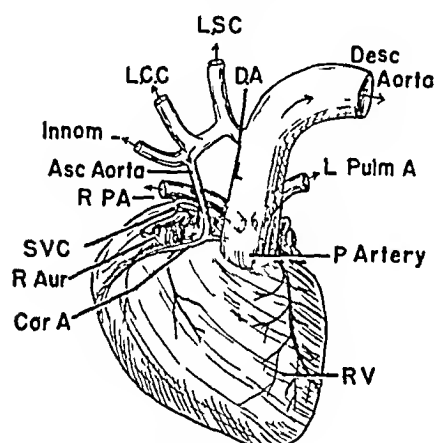
The left atrium was 0.3 cm wide and 0.7 cm long. The endocardium was finely trabeculated, and the wall was 0.3 cm thick. The left auricle was small and well formed. Entering the left atrium were two right and three left pulmonary veins. The upper right vein entered the atrium in the usual way. The lower right vein entered through a common orifice shared by the uppermost of the three left veins. The middle left vein entered the atrium independently in the same manner as the upper right. The lowermost left vein, having the same caliber as the others up to the wall of the atrium, entered by means of a pinpoint orifice. There was no trace of a mitral valve or of the left ventricle. The aorta began, without a trace of aortic valve leaflets or any vestige of endocardial cushions, at the level of the origin of the coronary arteries. The right coronary artery ran between the right auricle and the right ventricle and descended along the right border of the latter. The left coronary artery curved around the base of the pulmonary artery and divided into two branches at the level of the left auricle, supplying, respectively, the left border and the posterior aspect of the right ventricle. The ascending portion of the aorta was 0.2 cm wide externally, and its intimal surface was smooth. Its origin was almost directly behind the base of the pulmonary artery. From there it ran, with a slight obliquity, to the right and upward. One and one-half centimeters from its origin, it gave rise to the innominate artery, which was 0.4 cm wide, then to the left common carotid artery, 0.3 cm wide and then,

⁷ Popjak, G. J. Path. & Bact. 54: 67, 1942.

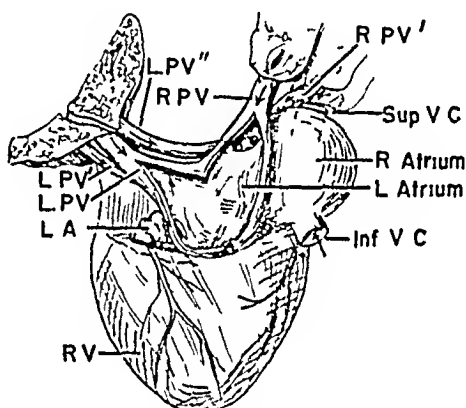
0.6 cm beyond, to the left subclavian artery, also 0.3 cm wide. Just beyond this it joined the ductus arteriosus and the descending aorta. Only beyond the ductus did the aorta broaden considerably, reaching a diameter of 0.7 cm (1.7 cm internal circumference), approximately the size of the pulmonary artery. The intercostal arteries arose in the usual manner from the thoracic aorta.

The lungs were buoyant, and each had the usual number of lobes. The thymus weighed 12 Gm and the spleen 15 Gm. The stomach, the duodenum and the small and large intestines were not unusual. The liver was dark brown and weighed 150 Gm. The pancreas, the adrenal glands and the kidneys were of the usual size. The pelvic organs were not remarkable. The brain weighed 350 Gm and presented nothing of note.

Microscopically, there was no evidence of inflammatory reaction in the heart. The area of junction between the right ventricle and the two atriums was occupied by hyalinizing fibrous connective tissue. There was no scarring in the adjacent myocardium or elsewhere.



ANTERIOR



POSTERIOR

Anterior view of the heart. *RV*, right ventricle, *Cor A*, right coronary artery, *R Aur*, right auricle at margin of right atrium, *SVC*, superior vena cava, *R PA*, right branch of pulmonary artery, *Innom*, innominate artery, *LCC*, left common carotid artery, *LSC*, left subclavian artery, *DA*, ductus arteriosus, *Desc Aorta*, descending aorta, *L Pulm A*, left branch of pulmonary artery, *P Artery*, pulmonary artery.

Posterior view of the heart. The left atrium is open. There is neither mitral valve nor left ventricle. *RV*, right ventricle, *LA*, left auricle, *LPV*, left middle pulmonary vein, *LPV'*, left lower pulmonary vein with pinpoint orifice in atrium, *LPV''*, left upper pulmonary vein, crossing to the right, to enter the atrium in a common orifice shared with the lower right pulmonary vein, *RPV*, upper right pulmonary vein, *RPV'*, lower right pulmonary vein, *Sup V C*, outline of superior vena cava on anterior aspect of right atrium.

In a preparation of tissue taken through the wrinkled portion of the ductus arteriosus, the intima was uneven, broad and loose. The thickening was due to an increase of both elastic and collagenous fibers. The media was uneven and, in places, narrow. The elastic laminae were scanty and uneven and were often interrupted by an increase of collagenous connective tissue. The adventitia was broader than the media and narrower than the intima. It was composed of heavy laminae of elastic fibers interspersed with collagenous fibers. Preparations of the aorta were not remarkable.

In the lungs the interalveolar capillaries were markedly distended with blood. The connective tissue of the bronchial walls was slightly increased and sparsely infiltrated with small round cells, large mononuclear cells and polymorphonuclear leukocytes. The bronchial capillaries were markedly distended with blood. The lumens of the bronchial veins were considerably widened and their walls thickened. The pulmonary veins, especially with the Van Gieson stain, were the most conspicuous feature of the preparations of the lungs. They were extraordinarily enlarged, and their walls were greatly thickened. The bulk of the thickening was composed of many laminae of elastic fibers between the slightly thickened intima and the slender adventitia. Compared with the veins, the pulmonary arteries were inconspicuous. In the arteries, the intima was slender, the media thickened slightly and the adventitia thickened and hyalinizing. In most instances the adventitia equaled or exceeded the media in thickness. Only vascular congestion was noted in the other organs.

Anatomic Diagnosis—Anomaly of the heart: absence of the left ventricle and of the mitral and aortic orifices, hypoplasia of the aorta, patent ductus arteriosus, hypertrophy of the right ventricle and of pulmonary and bronchial veins, passive congestion of viscera.

COMMENT

Any comment as to the mode of origin of the curious defects described is apt to arouse controversy without giving material aid, hence, none will be ventured. Concerning the circulation of the blood, this may be said. The dead end in the left atrium was apparently compatible with life for a few hours. Consequently there must have been some return of blood from the left side of the heart to the right. In view of the enlargement of the bronchial and pulmonary veins it seems safe to assume that there was a communication between the two systems. Even normally "a considerable quantity of the blood which is carried to the lungs through the bronchial arteries is returned to the left side of the heart through the pulmonary veins"⁸. In addition, there was a tidal flowing of blood back and forth through the lungs, with all the enlarged pulmonary veins being used as a pulsating expansion chamber. In each systole of the ventricle this venous expansion chamber was distended by aerated blood. In each diastole the elastic retraction of the heavy walls of these veins forced the aerated blood back into the pulmonary and bronchial capillaries, where it was mixed with the nonaerated blood coming through the pulmonary arteries. Of course, the blood in the systemic circulation was even less aerated than was this mixture, for it consisted largely of blood that had passed directly from the right atrium and ventricle into the aorta and great vessels without having entered the lungs at all. Considering the feeble aeration of this system, one finds it remarkable that the infant survived as long as she did.

⁸ Gray, H. The Anatomy of the Human Body, ed. 24, edited by W. H. Lewis, Philadelphia, Lea & Febiger, 1943, p. 669.

IN VITRO EFFECT OF PENICILLIN ON ENDAMOEBA HISTOLYTICA

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STUDIES on *Endamoeba histolytica*¹ have been an important activity of the Department of Parasitology and Bacteriology of the Michael Reese Hospital since the epidemic of amebic dysentery that occurred in Chicago in 1934. When a member of the department began to culture *Penicillium notatum* and harvest the crude penicillin, it was but natural that its effect should be tried on *E. histolytica* as well as on various bacteria. Observations were made on wet mounts of *E. histolytica* to which penicillin was applied directly and on cultures of the parasite treated with serial dilutions of the crude penicillin. No inhibitory action on wet preparations or on cultures of *E. histolytica* was observed. The study was temporarily discontinued because it did not appear feasible to use crude penicillin, and the purified product was unavailable.

In the latter part of 1944 a symposium on amebiasis was given at the Royal Society of Tropical Medicine and Hygiene². The participants unanimously agreed that there was urgent need for investigations of new chemotherapeutic agents in the treatment of amebiasis, because none of the drugs in current use was without toxic properties, and none assured cure. Hargreaves,³ Manson-Bahr⁴ and Willmore⁵ had independently used penicillin in treatment in cases of refractory amebiasis and had noted that clinical improvement of the patients was spectacular but that their stools still contained *E. histolytica*, and that when peni-

This study was aided by a grant from the Committee on Scientific Research of the American Medical Association.

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1 Howell, K M. Proc Inst Med Chicago **12** 193, 1938. Howell, K M, and Knoll, E W. Am J Dis Child **61** 54, 1941. Knoll, E W, and Howell, K M. Am J Clin Path **15** 178, 1945. Howell, K M, and Knoll, E W. J Am Women's A **1** 203, 1946.

2 Adams, A R D. Tr Roy Soc Trop Med & Hyg **38** 237, 1945, Lancet **2** 752, 1944.

3 Hargreaves, W H. Tr Roy Soc Trop Med & Hyg **38** 244, 1944.

4 Manson-Bahr, P. Tr Roy Soc Trop Med & Hyg **38** 251, 1944.

5 Willmore, J G. Tr Roy Soc Trop Med & Hyg **38** 257, 1944.

cillin therapy was discontinued, the clinical symptoms recurred. In a later publication Hargreaves⁶ reported the use of penicillin and succinyl sulfathiazole followed by a specific drug as the most effective treatment in refractory cases of amebiasis. In this report he stated, without submitting data, that neither sulfathiazole nor penicillin was effective for amebas in the laboratory.

We recalled our early attempts to inhibit amebas by the addition of crude penicillin *in vitro* and decided to repeat and expand our initial experiments, using purified penicillin.

DIRECT MICROSCOPIC PROCEDURE

Two similar wet preparations of feces teeming with motile *E histolytica* were prepared for microscopic examination. A drop of penicillin was added to one mount and a drop of saline solution to the other so that the concentrations of the two were similar. Different portions of the mounts were examined to ascertain that approximately equal numbers of motile amebas were present in each mount. These preparations were observed on the warm stage at five minute intervals for two or three hours. The amebas remained motile, and no change in their activity was noticed. On a number of occasions one typical ameba of the penicillin-treated wet mount was kept in the field and watched for an hour. At the end of the hour this ameba always retained its motility, pseudopods were still being extruded, and morphologically it appeared normal. This direct application of solutions containing from 5 to 10,000 Oxford units of penicillin per cubic centimeter to specimens of stools containing motile amebas and of twenty-four hour positive cultures was repeated a number of times, with negative results.

CULTURAL PROCEDURE

The effect of penicillin on cultures of *E histolytica* was tested by making serial dilutions of a penicillin standard (100, 500, 1,000 and 5,000 Oxford units per cubic centimeter) and adding known quantities per cubic centimeter to the liquid portion of Cleveland's⁷ medium (1 part of serum to 6 parts of isotonic solution of sodium chloride, p_H 7 to 7.2). The penicillin levels of the finished medium corresponded roughly to blood levels obtained in patients treated for various bacterial infections, i. e., the levels varied from a trace, 0.03 unit, to 20 units or more. Each tube, containing 4.5 cc of liquid culture medium, was inoculated with 0.5 cc of a uniform suspension of emulsified stool or with 0.5 cc of a uniform suspension of stool culture. The cultures were incubated at 37°C and examined at intervals of twenty-four, forty-eight and seventy-two hours for motile amebas, for level of penicillin, for proportion of gram-positive to gram-negative organisms and for hydrogen ion concentration (p_H). A number of strains of *E histolytica*, including one from the Army, and diverse types of stool specimens were cultured, namely, fecal material in which cysts of *E histolytica* predominated, fecal material containing trophozoites and cultures and subcultures teeming with motile amebas. For comparison, similar tests were run, in which sulfadiazine, carbarsone, or chiniofon was substituted for penicillin. Sulfadiazine was used on the supposition that it would suppress gram-negative flora, in contrast to the suppression of gram-positive flora by penicillin, and thereby indicate the effect of different

⁶ Hargreaves, W. H. *Lancet* 2: 68, 1945.

⁷ Cleveland, L. R., and Collier, J. *Am J Hyg* 12: 606, 1930.

types of bacterial flora on the viability of amebas Carbarsone was included as a control because it was the drug most commonly used by the staff of the Michael Reese Hospital in the treatment of amebiasis Cliniofon was used because it acts directly on amebas as demonstrated by its general use in retention enemas Control cultures of *E. histolytica* were always included to prove the viability of the strain used In some series of cultures the penicillin level decreased until there was only a trace at the end of twenty-four or forty-eight hours Because of this, a series of cultures was set up in which the level was maintained at a constant by adding penicillin at intervals and checking the level Some of the drugs used are more effective in an alkaline medium, and one set of cultures was alkalinized, pH 7.5, and studied for divergent results

Effect of Penicillin on Cultures of Four Strains of Endamoeba Histolytica

| | Tube 1* | Tube 2 | Tube 3 | Tube 4 | Tube 5 | Tube 6 | Control Tube |
|------------------------|----------|----------|----------|----------|----------|----------|--------------|
| Strain 1 | 0.4 unit | 1 unit | 2 units | 3 units | 4 units | 6 units | 0 |
| 24 hr. reading | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| 48 hr. reading | ++ | ++ | ++ | ++ | 0 | 0 | ++ |
| Strain 2 (Army strain) | 2 units | 5 units | 10 units | 15 units | 20 units | 30 units | 0 |
| 24 hr. reading | ++ | ++ | ++ | ++ | ++ | 0 | ++ |
| 48 hr. reading | ++ | ++ | ++ | ++ | 0 | 0 | ++ |
| Gram positive flora | 75% | 50% | 40% | 35% | 25% | 50% | 50% |
| pH | 6.5 | 7.0 | 7.0 | 6.5 | 6.5 | 6.5 | 6.5 |
| Strain 3 | 4 units | 10 units | 20 units | 30 units | 40 units | 60 units | 0 |
| 24 hr. reading | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| 48 hr. reading | ++ | ++ | ++ | ++ | ++ | 0 | ++ |
| Gram positive flora | 50% | 60% | 50% | 50% | 50% | 10% | 50% |
| pH | 7.0 | 6.5 | 7.0 | 6.5 | 6.5 | 6.5 | 6.5 |
| Strain 4 (cysts) | 4 units | 10 units | 20 units | 30 units | 40 units | 60 units | 0 |
| 24 hr. reading | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| 48 hr. reading | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| Gram positive flora | 10% | 10% | 10% | 30% | 40% | 40% | 60% |
| pH | 7.0 | 6.5 | 7.0 | 6.5 | 6.5 | 6.5 | 6.5 |

* Each tube contained 4.5 cc. of diluted serum and 0.5 cc. of amebic inoculum. The given amount of penicillin, in Oxford units per cubic centimeter, was added to the medium before addition of the inoculum.

++ = motile amebas, + = a few motile amebas, ± = precysts, 0 = negative for amebas

The table illustrates the results of subjecting four strains of *E. histolytica* to various concentrations of penicillin. Only an occasional inhibitory effect was observed, regardless of whether the inoculum was feces, culture or subculture, or whether it contained cysts or trophozoites, as long as it was rich in amebas. Similar sets of cultures treated with sulfadiazine did not show any effect on the viability of the amebas. Cliniofon and carbarsone tended to inhibit the growth of *E. histolytica*. Alkalinizing the cultures had no apparent effect on the amount of growth. The set of tests in which the penicillin level was maintained at a constant showed no appreciable effect on the viability of the amebas. The proportion of gram-positive to gram-negative flora in the amebic cultures had little uniformity, regardless of the addition of either penicillin or drugs. However, in examining smears, it was noted that there were always a few of those that had been subjected to penicillin in which there was a suppression of

gram-positive organisms and a predominance of gram-negative organisms even up to 70 to 95 per cent. The sulfadiazine-treated cultures reversed these findings. Carbarsone and chiniofon were irregular in their effect on the fecal flora. In all cultures the p_H varied from 6 to 7.5, a range in which *E. histolytica* is viable.

RESULTS AND CONCLUSIONS

Wet mounts of emulsions of feces teeming with *E. histolytica* subjected to penicillin varying in concentrations from 5 to 10,000 units per cubic centimeter still contained motile amebas at the end of three hours.

Cultures of *E. histolytica*, either cysts or trophozoites, were in most instances unaffected by penicillin even when the penicillin levels were maintained as high as 30 Oxford units per cubic centimeter for forty-eight hours.

Penicillin tended to suppress gram-positive bacteria in cultures.

The fecal flora, either normal or abnormal, had no apparent effect on *E. histolytica*.

Variation in the hydrogen ion concentration of cultures from p_H 6 to p_H 7.5 did not influence the viability of *E. histolytica*.

In vitro studies indicated that penicillin had little or no effect on either the trophozoites or the cysts of *E. histolytica*. By inference it seems probable that the improvement observed in the clinical symptoms of patients with amebiasis under penicillin therapy must be due to the action of the drug on the secondary bacterial invaders of the tissues.

SOME EFFECTS OF PROLONGED MASSIVE ESTROGEN TREATMENT ON THE RAT

With Special Reference to the Thymus

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THE CYSTIC epithelial structures developing in the thymus after prolonged massive treatment with estrogenic substances were reported in 1941 by Ross and Korenchevsky.¹ Their report was confirmed by me in 1944.² An extension of these observations, described in the present account, affords additional information on the development and the structure of these abnormal growths.

The typical histologic pattern of the thymus of a young and healthy animal, with its sharp demarcation into cortex and medulla, is readily modified by a wide variety of conditions. The so-called age involution or, in the younger animal, the accidental involution resulting either from pathologic conditions or from a variety of stimuli (Selye³) causes pronounced and characteristic atrophic changes in the appearance of the thymus. The distinction between cortex and medulla is lost, lymphocytes disappear, and the parenchyma is invaded and replaced by connective tissue.

It has been demonstrated repeatedly that injections of estrogens will produce rapid involution of the thymus. The nonspecificity of the many agents or factors producing thymic atrophy and the uniform appearance of the involuted thymus, irrespective of its cause, are well known. It was thus of special interest to discover that conspicuous epithelial cysts could be produced in an atrophic thymus simply by increasing the dose of the estrogen and extending the treatment.

EXPERIMENTAL PROCEDURE

Twenty-three adult albino rats (Sprague-Dawley) were used. Twelve were treated continuously for periods varying from one month to ten and one-half months with estradiol dipropionate, which was injected weekly in doses of either 0.1 or 0.2 mg (0.1 mg in 0.1 cc of sesame oil). The remaining 11 animals either were left untreated or were treated with a volume of sesame oil equivalent

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The estradiol dipropionate used in this investigation was supplied by Ciba Pharmaceutical Products, Inc., through Dr. Ernst Oppenheimer.

1. Ross, M. A., and Korenchevsky, V. *J. Path. & Bact.* **52**: 349, 1941.

2. Plagge, J. C. *Anat. Rec.* **89**: 537, 1944.

3. Selye, H. *Brit. J. Exper. Path.* **17**: 234, 1936.

to that administered to the experimental rats. As far as possible the direct comparisons of test and control animals were made between litter mates. The ages at the beginning of the injection periods ranged from 2 to 6 months. All of the animals except 2 were females, 2 of the test females and 1 of the controls were spayed at the time treatment was begun. However, neither sex nor castration modified the effects of excessive estrogen treatment.

During the course of the treatment the animals were weighed once a week, and any changes in general health or in color and texture of hair were noted. At autopsy the thymus was removed quickly, weighed in a milligram torsion balance and fixed immediately in Zenker's solution to which solution of formaldehyde U S P had been added in the concentration of 5 per cent. Tissues were embedded in paraffin, sectioned at 8 microns and stained with hematoxylin-eosin-azure II.

RESULTS

As in the experiments described in previous reports, in which three to four months was the extent of treatment with massive doses of estrogens, conspicuous large and small epithelium-lined cysts appeared in the thymus (fig 1). Usually the cells lining the lumens were irregular in size and shape, but occasionally they were columnar or cuboidal and fairly well organized, resembling either a simple or a stratified epithelium (figs 2 and 3).

A striking characteristic of the cyst was the material contained within its lumen. There was an apparent secretion that stained in a variety of colors, from pale pink, through various shades of blue, to a deep purple. It was common for two colors of secretion to be present within the same cyst. When this occurred and the cysts were numerous, their follicular appearance was suggestive of the thyroid gland.

Actual evidence that this material was being secreted into the follicle was never obtained in fixed preparations. However, lining the cysts were numerous cells which at their distal ends had cytoplasm that took the same red and blue colors as the cyst cavity. There were also occasional small solid nests or cords of cells with colored cytoplasm that were not associated with an actual cyst. This observation suggests the beginning of a new cyst. In addition, there were occasional isolated single epithelial cells containing the same colored material.

In some cysts filled with a pink secretion numerous blue droplets were present. It is possible that the blue material had been secreted more recently.

When the cysts were small, they tended to be isolated, when they were large, they were likely to be interconnected by cords or tubes. They always ended blindly and were surrounded either by the parenchyma or the connective tissue of the thymus.

Debris, consisting primarily of degenerating cells, was frequently present within the secretion of the cysts. This intrafollicular material appeared to be of epithelial origin, although degenerating lymphocytes

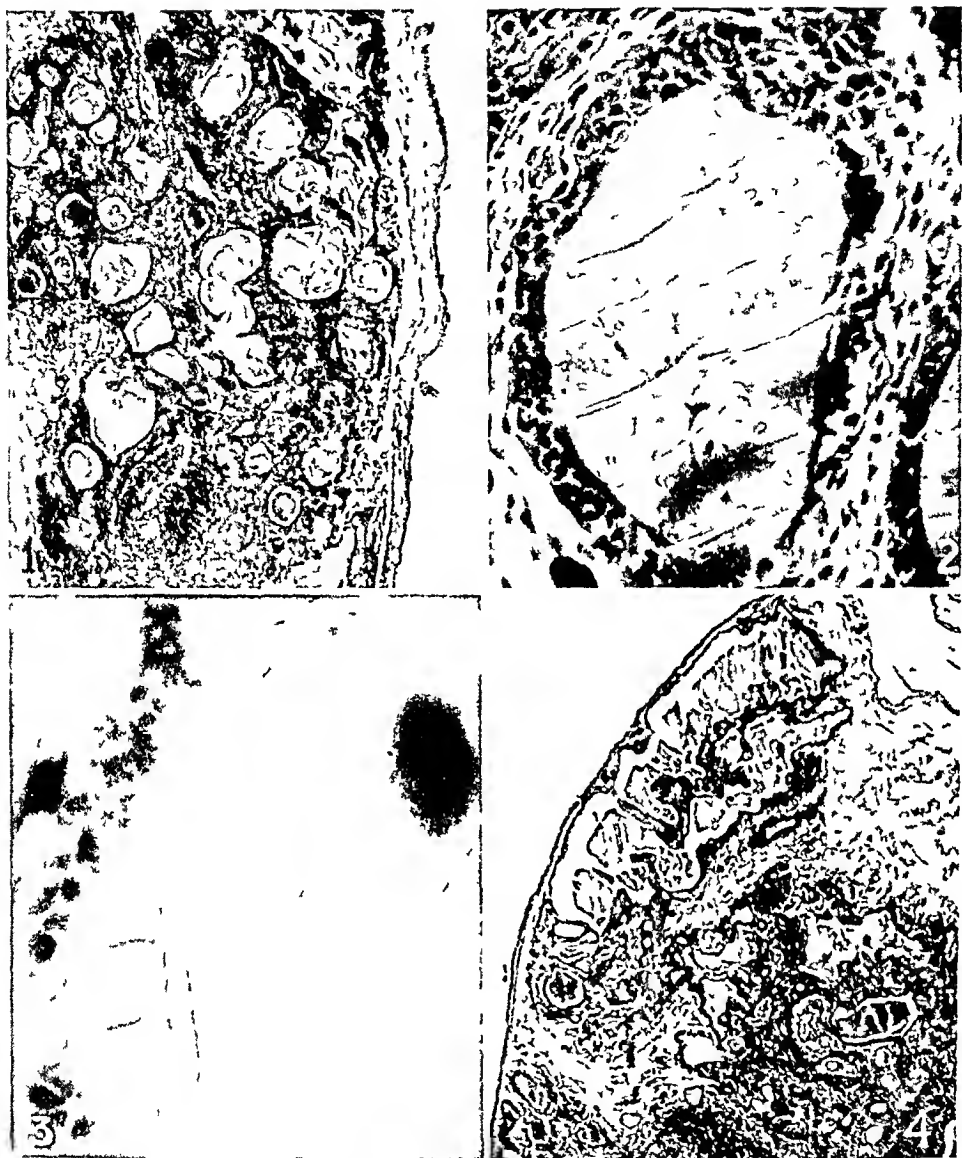


Fig 1 (rat treated by weekly injection of 0.2 mg of estradiol dipropionate for ten and a third months)—Epithelium-lined cysts pervade the entire area of the section of thymus. One remaining island of lymphocytes appears near the bottom of the figure $\times 57$.

Fig 2 (rat treated by weekly injection of 0.2 mg of estradiol dipropionate for nine months)—Small thymic cyst showing a tendency toward irregular stratification of epithelium $\times 382.5$.

Fig 3 (rat treated by weekly injection of 0.1 mg of estradiol dipropionate for four months)—Epithelium of a thymic cyst. Note the distention within the cytoplasm at the lower left side $\times 875.5$.

Fig 4 (rat treated by weekly injection of 0.2 mg of estradiol dipropionate for ten and a third months)—Development of a large thymic cyst. Portions of epithelium are being pinched off into the cavity. Fluid and cellular debris can be seen within the cysts in this figure and also in the others $\times 57$.

of the thymus were occasionally observed. In certain favorable sections desquamation of the outer layer of the stratified epithelium was seen. In other instances, in which cyst formation was extensive, small islands of epithelium appeared to have been pinched off and set free within the cavity of the cyst (fig. 4).

The relative size and the number of cysts were approximately proportional to the length of treatment. The thymuses of animals that had been treated with estrogens for ten months were depleted almost completely of lymphocytes, and the epithelial neoplasms were much more numerous than in those with only four months' injection. However, the total size of the thymus was further reduced by approximately 50 per cent in the rats that had been treated for the longer period. Thus it was not possible to produce a true tumor in the thymus by extending the period of treatment to approximately two and a half times the duration reported previously. The pathologic condition was exaggerated, but the thymus was reduced in size.

It has been reported by Marine⁴ that epithelial structures resembling those described here are found occasionally in the thymuses of most animals, most commonly in that of the dog. Ross and Korenchevsky¹ have implied that such structures were rare in their control rats. Only in 3 of 81 normal controls did the thymus contain a "few small undeveloped nests of epithelial cells." Similarly, Selye,³ who observed the induction of tubular epithelial structures in the thymus of the rat forty-eight hours after subcutaneous treatment with morphine, has never seen such formations in the thymus of a normal rat. In the present series, in which 11 animals served as either untreated or sesame oil-treated controls, the thymuses of 6 animals were studied in serial sections. Small epithelial cysts could be found by careful searching in all the control thymuses examined. A few of these cysts approached the development of the smallest cysts found in the experimental animals (figs. 5 and 6). The injection of pure sesame oil had no influence on the occurrence of the structures.

In an attempt to determine the origin of the cysts a small series of animals was subjected to colchicine treatment following preliminary massive estrogen stimulation of one, two or three months' duration. A colchicine dose of 0.1 mg. per hundred grams of body weight of rat was injected eight hours before the rat was killed and examined. There was no apparent accumulation of mitotic figures following the terminal application of colchicine.

The first indication of cystic development was usually seen in the medulla of the thymus (fig. 7). In the estrogen-treated animals solid cords of epithelial cells pervaded this area, becoming increasingly

⁴ Marine, D. in Cowdry, E. V. *Special Cytology*, New York, Paul B. Hoeber, 1928, vol. 1, sect. 17, p. 549.

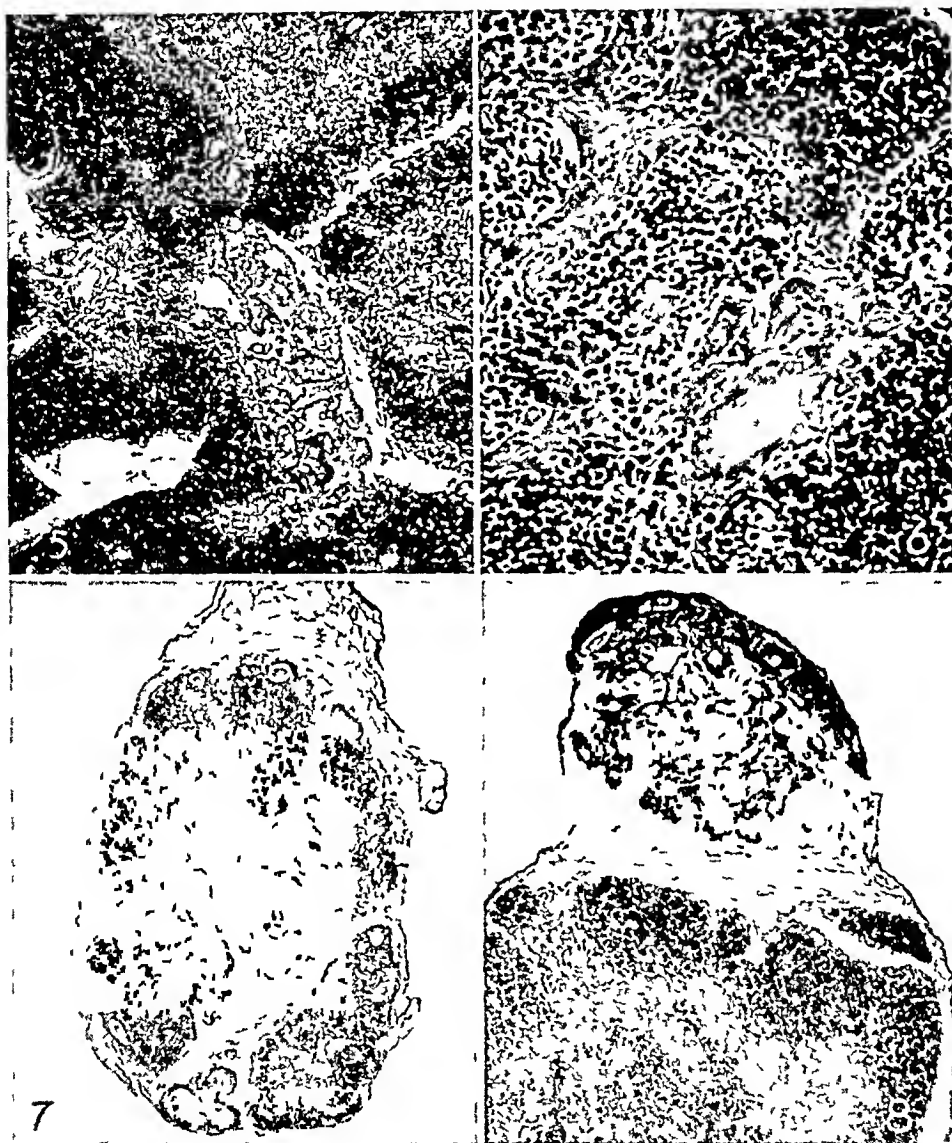


Fig 5 (control treated by injection of sesame oil for four months) —Small cysts in an otherwise normal-appearing thymus $\times 57$

Fig 6 (control given no treatment) —The epithelial cysts within the parenchyma of the thymus are similar to the larger cysts of the estrogen-treated animals $\times 229.5$

Fig 7 (rat treated by weekly injections of 0.2 mg of estradiol dipropionate for three months) —The early development of epithelial cysts is primarily in the medullary portion of the thymic lobule $\times 57$

Fig 8 (rat treated by weekly injection of 0.2 mg of estradiol dipropionate for three months) —Section through both lobes of the thymus, the smaller area, with many cysts, is from the cranial end of one lobe, the larger area, from the other lobe, represents a more caudal region and contains only a few cysts and epithelial cords $\times 57$

prevalent as an anastomosing network, within the meshes of which lymphocytes were trapped. Concomitant with the loss of distinction between cortex and medulla, the cords invaded the area of the cortex. Simultaneously a few small cysts frequently appeared in the connective tissue, entirely isolated from the parenchyma of the thymus. In the later stages the cords developed lumens, many of which ballooned into large spheric cysts.

If a large and growing cyst encountered any resistant structure, such as a large blood vessel, it usually forked and wrapped itself around the structure.

Serial sections of the thymus showed that the cysts did not occur uniformly throughout the organ. Marine⁴ observed that the cranial extremities of the lobes were the favored location for their origin. In order to test this conclusion in the present experiment care was exercised to distinguish the cranial and the caudal ends of the thymus during the preparation of the sections.

Cysts occurred throughout the thymus but were found more often at the ends of the organ. Usually the cranial end had more cysts than the caudal end. Figure 8 shows a cross section through both lobes of the thymus. The two lobes were oriented in such a way that the section includes an area from the cranial end of one lobe and from a more central region of the other lobe. The smaller area at the top of the picture, showing many cysts, is from the cranial end of one lobe. The larger area, from the other lobe, represents a more caudal region and contains only a few cysts and epithelial cords. It is well infiltrated with lymphocytes, and the distinction between cortex and medulla is typically sharp.

In the most severely altered thymuses only traces of lymphatic areas remained. These were usually located peripherally in the lobes, but now and then a resistant nodular island of normal-appearing thymus tissue remained (fig. 1).

Although this report is intended to deal principally with thymic effects, other extragenital alterations were observed as a result of the long-continued massive injections of the estrogen. These will be treated briefly in this report.

The more prolonged periods of injection were frequently terminated prematurely by the occurrence of pyometra and occasionally by infections of the respiratory tract and other complications. Injections continued for three or four months stimulated the uterus without causing an infection but severe pyometra developed when the injections were continued for several more months. The condition apparently came on abruptly, judged from the sudden and rapid decrease in body weight and the general weakened condition of the animal. Although the injections were to have been continued for at least one year, ten and a

half months was the longest period of treatment possible under these conditions. Because of the high frequency of this infection and because of its possible secondary influence on the involution of the thymus, a few animals were killed while still healthy. The induction of pyometra following estrogenic treatment has been observed by numerous investigators (Kaufmann and Steinkamm⁵, Gardner⁶, Hale and Weichert⁷, Nelson⁸).

The body weights of the estrogen-treated animals, taken routinely at weekly intervals, were appreciably less than those of their litter mate controls. The weight of the estrogen-treated animal usually remained at a plateau during treatment whereas that of the control gradually increased. This suppression of growth confirms earlier reports by Forbes,⁹ Hooker and Pfeiffer¹⁰ and Bogart, Lasley and Mayer¹¹.

During the course of treatment the fur of the rats was observed for any alterations. Forbes,¹² several months after subcutaneous implantation of pellets of a crystalline estrogen, noticed partial pigmentation of the fur and partial alopecia. In the present experiment no color change of hair attributable to estradiol dipropionate was noted although the lengths of treatment and the amounts of the estrogen given corresponded in general to those employed by Forbes.¹² However, a brownish tipping of the hair, particularly on the scruff of the neck, which appeared quite similar to that described by Forbes,¹² developed in a few rats suffering from respiratory tract or other infection whether or not they were given injections. It is not assumed that this coloring is in any way related to the overdose of estrogen, since healthy rats had a snowy white coat after eight or ten months of continuous treatment. The discrepancy in results, since the dosage was comparable, may be due to differences in strains of rats or to a more constant rate of absorption with implantation of pellets. Interference with hair growth, however, was definitely observed. In shaved areas the hair grew out at about one-half normal rate in animals receiving injections. This substantiates an observation made in rats by Hooker and Pfeiffer¹⁰ and a recent report by Williams, Gardner and DeVita¹³ of experiments in which estrone was applied locally on dogs.

5 Kaufmann, C, and Steinkamm, E. *Arch f Gynak* **162** 553, 1936

6 Gardner, W U. *Cancer Probl, Symposium, Science*, 1937, supp, vol 85, p 67

7 Hale, H B, and Weichert, C K. *Proc Soc Exper Biol & Med* **55** 201, 1944

8 Nelson, W O. *Yale J Biol & Med* **17** 217, 1944

9 Forbes, T R. *Endocrinology* **30** 761, 1942

10 Hooker, C W, and Pfeiffer, C A. *Endocrinology* **32** 69, 1943

11 Bogart, R, Lasley, J F, and Mayer, D T. *Endocrinology* **35** 173, 1944

12 Forbes, T R. *Endocrinology* **30** 465, 1942

13 Williams, W L, Gardner, W U, and DeVita, J. *Endocrinology* **38** 368, 1946

Daily vaginal smears demonstrated that estrogen treatment produced a three to four week period of diestrus, followed by continuous estrus. Occasionally at the beginning of treatment a few days of estrus occurred before the diestrus period. The prolonged diestrus period following excessive administration of estrogen in normal female rats was first observed by Selye, Collip and Thomson¹⁴ and is associated with large transitory corpora lutea in the ovary.

During the process of obtaining vaginal smears 4 of the estrogen-treated females assumed a momentary lordosis, similar to the mating response. One female responded this way on seventeen of thirty days.

COMMENT

In this experiment an attempt was made to produce a true tumor or cancer in the thymus of a rat by giving massive doses of estrogen for a period of ten and a half months. At the end of this time there was no indication of a carcinoma. On the contrary, the thymus was further reduced in size by involution. But the atypical cystic growths, observed after treatment of three to four months, were considerably more extensive. Inflammatory developments in the uterus, terminating in severe pyometra and other complications, precluded further extension of treatment.

While the experiments reported here confirm the previously published contention that excessive estrogen treatment is responsible for the development of an extensive growth of epithelial cords, tubes and fluid-filled sacs in the thymus, a thorough histologic analysis of the thymuses of untreated litter mate controls revealed in a majority of cases similar, though much smaller, epithelial structures.

It appears that estrogens influence the thymus in two ways. In the first place, they cause typical involution—decrease in size and loss of lymphocytes. Continued treatment produces the extensive epithelial cysts. Estrogens are thus capable of producing an atrophic condition in the thymus and subsequently inducing active growth of an epithelial nature.

Although it has not been possible to determine the origin of the epithelial network, it is probable that the reticulum is involved, especially since the structures are usually first detected in the medulla of the parenchyma and rarely in the connective tissue. The epithelial nature of these pathologic structures recalls the embryologic origin of the thymus from the pharyngeal pouches. Hassall's corpuscles are so rare and so poorly developed in the thymus of the rat that it is unlikely that they are associated with abnormal growths. However, Steiner,¹⁵

14 Selye, H., Collip, J. B., and Thomson, D. L. *Proc Soc Exper Biol & Med* 32 1377, 1935,

15 Steiner, P. E. *Proc Soc Exper Biol & Med* 49 62, 1942

who induced similar epithelial hyperplasia in the thymus of the guinea pig by direct implantation of pellets of methylcholanthrene, suggests that at least some of the cysts produced in the thymuses of his guinea pigs were actually enlarged Hassall's bodies

SUMMARY

Extensive epithelial cords and cysts, containing fluid, have been produced in the thymus of the rat after prolonged massive treatment with estradiol dipropionate. Tumors failed to develop even after ten and a half months of continuous injections. Further treatment had to be abandoned because of the development of pyometra, infections of the respiratory tract and other complications leading to death. Cystic structures were most pronounced at the ends of the thymus, particularly at the cranial extremity.

Small epithelial structures, similar to the larger ones of the test animals, were found in the majority of the controls,

Body weight, hair growth and the estrus cycle were also modified by the prolonged and excessive treatment with estrogen.

MEDIAL CALCIFICATION OF ARTERIES OF INFANTS

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SCHENECTADY, N. Y.

THE GENESIS of arteriosclerosis has occupied the minds of scientific workers for more than a century. Among the various theories on this subject, none has found wider acceptance or remained in general favor over a longer period than the senescence theory. In recent years more and more investigators have refused to accept arteriosclerosis as a natural involutionary process of aging and have looked on it as a disease initiated by some form of injury of the arterial wall followed by sequence of changes.

Arteriosclerosis occurring in infants, although rare, is of considerable interest. While the arteriosclerosis of the adult very likely is in most instances a complex product brought about by action of several agents to which the vessels were subjected in the course of life, it is obvious that less complicated causal and developmental conditions prevail in infants showing arteriosclerosis.

Following is a report of extensive medial calcification of arteries in an infant 10 weeks old. Data on similar lesions in infants are summarized in the accompanying table.

REPORT OF A CASE

A 10 week old baby girl was admitted to the hospital on September 8 because of general debility and wasting.

The infant was born at full term to healthy white parents. The father was 20 and the mother 17 years of age. The Wassermann reaction of the blood of each parent was negative. The mother had been well during pregnancy and had not received vitamins or any form of medication. The labor and the delivery were uneventful. At birth the infant measured 47 cm. in length and weighed 2.5 Kg. No cyanosis, convulsions or abnormalities were noted. The hospital record showed that the infant took the feedings well. The weight, after the usual decline in the first few days, reached the initial birth weight on the seventh day. The mother and the baby left the hospital after eight days. The history of the child's development from the time of discharge until the time of reentry is incomplete. On questioning, the mother stated that the infant presented no difficulties in the first five weeks. It was fed on a formula of evaporated milk and water (1:3). The bowel movements were normal, and the baby cried little. In the sixth week a physician was consulted, who suggested that 5 drops of percomorph liver oil be added once a day to the feeding formula. In the following weeks the baby "did not take the bottle well," leaving about half of it. A record of the weight

From the Department of Pathology, Ellis Hospital

could not be obtained. The week before admission the mother noticed that the baby seemed to lose weight. It would lie for hours in the crib without making a noise, as if it were too weak to move or cry. On September 7 the baby was taken to the office of a local physician. Immediate hospitalization was advised.

When admitted to the hospital the next day the infant was cyanotic and undernourished, and the turgor of tissue was poor. The anterior fontanel was soft. The extremities were cold and clammy, the respirations, shallow and labored. The temperature was 97.4 F. The infant was placed in an oxygen tent and a 5 per cent dextrose solution administered by clivsis. Its color did not improve, and death occurred three hours later.

Necropsy (ten hours after death).—The body was that of an emaciated infant girl of gracile build, 56 cm long and weighing 3.4 Kg. The skin of the face and the finger nails were deeply cyanotic. There were no external signs of congenital syphilis or rickets. The pleural cavities contained no free fluid, the lungs were crepitant, although of firmer consistency than normal. The thymus weighed 12 Gm and appeared normal. The pericardial cavity contained about 15 cc of a straw-colored fluid. The heart weighed 35 Gm. The right ventricle appeared dilated. The coronary arteries were prominent, rigid and tortuous. They cut with gritty resistance, and their lumens were scarcely visible. The epicardium was smooth, the myocardium, pale and of less firm consistency than normal. The valves and the endocardium showed no lesions. The foramen ovale was closed. The aorta, the pulmonary arteries and the carotid arteries appeared normal on gross examination. The liver extended 6 cm below the xiphoid process and on section revealed a mottled light and dark brown cut surface. The gastrointestinal tract showed no anomalies. The spleen, the pancreas, the adrenal glands, the kidneys, the urinary bladder and the pelvic organs were normal on gross examination. Likewise, the ribs, the vertebrae and the skull. The brain showed no lesions, and all the cerebral vessels were thin and delicate. The organs of the neck revealed no significant anomalies. The parathyroid glands occupied their normal position and were not enlarged. Unfortunately, the changes observed later microscopically, were not anticipated at the time of the autopsy and the vessels of the extremities were not examined.

Microscopic Examination.—Both coronary arteries and their main branches showed extensive changes, characterized by medial calcification, intimal proliferation and consequent narrowing of the lumens. The thickening of the wall was due to marked fibroblastic proliferation of the subendothelial layer of the intima. Immediately adjacent to it was a continuous ring of calcareous material, which stained deep bluish violet with hematoxylin-eosin. The calcareous ring varied greatly in thickness, in places it was thin (fig 1A), and in others it occupied nearly one half of the thickness of the wall. Calcification of the internal elastica was noted in some vessels, but the membrane still retained its acidophilic properties and could be identified as a thin line within the calcium strands. The outer layer of the media was thin. In some vessels its muscle fibers were well outlined, in others, fragmented. The only noteworthy change in the adventitia was the presence of small focal collections of lymphocytes and eosinophils in one coronary artery. The innermost layer of the intima appeared to be normal. In the sections of the myocardium there were focal areas in which the muscle fibers were replaced by fibrous connective tissue. Sections of the arch of the aorta showed no significant changes. Lesions similar to those encountered in the coronary arteries were found in the arteries of the larynx, the thyroid gland, the mesentery, the pancreas, the adrenal glands and the kidneys. In the spleen, one artery sectioned

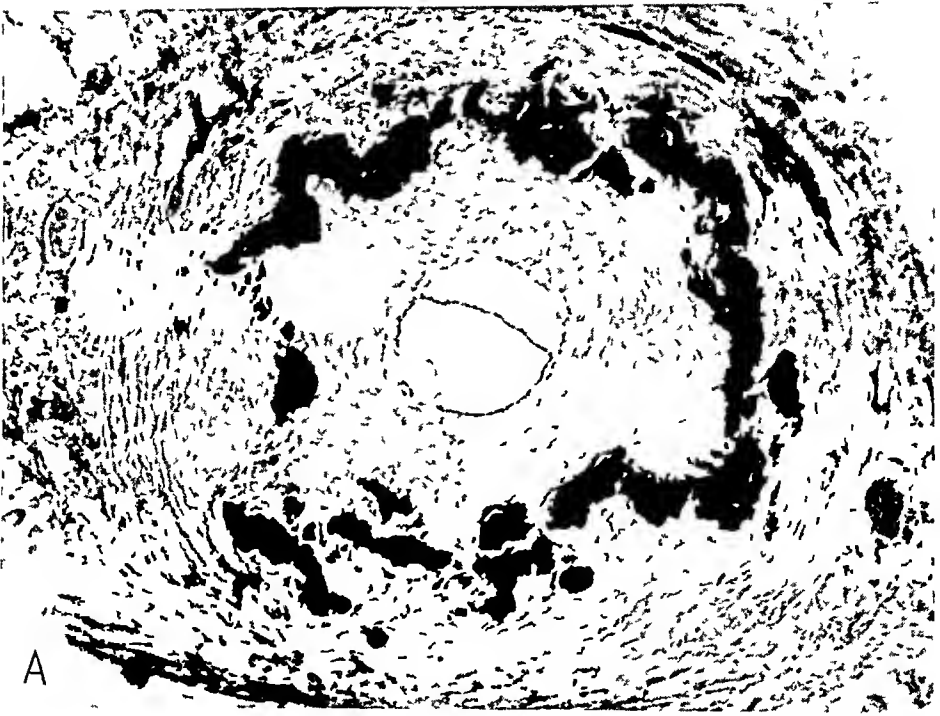


Fig 1—*A*, low power photomicrograph of the left coronary artery, showing calcium deposits in the media and fibroblastic proliferation of the intima *B*, splenic artery. Note the abrupt line between the normal and the involved portion of the arterial wall.

*Summary of Data on Fifteen Infants in Whom Medial Calcification of Arteries Was Observed**

| Author | Age | Sex | Arteries Involved † | | | | Calcium Deposits in Other Organs | Other Findings | Factors Stated by Authors to Be Responsible for Arterial Calcification |
|-------------------------------------|---------|-----|---------------------|-------------------|-------------------|-------------------|----------------------------------|---|---|
| | | | Pulmonary Arteries | Coronary Arteries | Cerebral Arteries | Visceral Arteries | | | |
| Durante ¹ | 17 days | ? | + | ? | ? | ? | 0 | Infection of navel, acute peritonitis | Intrauterine infection |
| Bryant and White ² | 6 mo | M | 0 | + | + | + | + | Congenital urethral dilatation, hydro-nephrosis | Renal disease |
| Surbeck ³ | 3 days | M | + | + | + | + | + | Fibrinous pericarditis, splenomegaly | Sepsis, nephritis, "calcium gout" |
| Verocay ⁴ | 5 mo | F | 0 | + | + | + | 0 | Congenital syphilis | Congenital syphilis |
| Jaffé ⁵ | 2 days | M | 0 | 0 | 0 | 0 | 0 | | Hydramnios, toxic factor of maternal origin |
| Hughes and Perry ⁶ | 7 wk | F | 0 | + | ? | ? | 0 | | Sepsis, dystrophic calcification |
| Ferrer ⁷ | 3 mo | M | 0 | + | + | + | 0 | Granuloma of navel, splenomegaly | Hydramnios, incomplete development of "ground substance" of arteries |
| Iff ⁸ | 1 day | M | + | + | + | + | 0 | Asclites | |
| Oppenheimer ⁹ ‡ | 6 mo | F | 0 | + | + | + | 0 | | |
| Oppenheimer ⁹ ‡ | 4 mo | F | 0 | + | + | + | 0 | | |
| Oppenheimer ⁹ ‡ | 11 days | F | + | ? | ? | ? | + | Nephritis (?) Congenital malformation of genitalia | Infection of urinary tract |
| Ross and Williams ¹⁰ | | ? | 0 | + | + | + | + | Bronchopneumonia | Hypervitaminosis D |
| Brown and Richter ¹¹ | 3 mo | M | 0 | + | + | + | + | | Alteration in calcium phosphorus metabolism, hypervitaminosis D(?) |
| Baggenstoss and Keith ¹² | 8 wk | F | + | + | + | + | 0 | | Disturbance of calcium metabolism, abnormality of arterial wall |
| Field | 10 wk | F | 0 | + | + | + | 0 | | Physicochemical or toxic changes in the intercellular matrix of arterial wall |

* The group does not include infants older than 12 months

† An interrogation sign indicates that the arteries were not mentioned by the author

‡ Oppenheimer reviewed 15 000 autopsy reports and found that arterial calcification had been noted in 3 infants

See footnotes on next page

showed a continuous ring of calcium in the media. In another artery the calcification of the media and the proliferation of the intima were present in about one half of the circumference of the vessel, while the other half appeared entirely normal. There was an abrupt line between the normal and the involved portion of the wall (fig 1B). A small artery in the pericapsular fat of the adrenal gland revealed large irregular deposits of calcium in the media and the intima separated by fibrous-like tissue. Only a small lumen remained. In sections of both lungs edema of the septums was noted. The branches of the pulmonary arteries showed scattered longitudinal streaks of calcareous deposits in the intercellular matrix along the surface of the elastic fibers of the media.

There was a moderate increase of the fat content of the cells of the liver, but no other abnormality was noted.

The parenchymal cells of the parathyroid glands were normal in size, shape and staining qualities and in relation to each other.

The basilar artery and the arteries of the circle of Willis were normal.

COMMENT

Fourteen reports of arteriosclerosis occurring in infants are found in the literature. A review of the cases shows that from the point of view of the morphology the lesions are similar if not identical in all. The inner and the middle third of the media are the site of predilection for the deposition of calcium, where it is intimately related to the internal elastic lamina and to the interstitial substance adjacent to this. Associated with the calcification of the media is fibroblastic proliferation of the intima with consequent narrowing or obliteration of the lumen. Specific degenerative changes (lipoid deposits, hyalinization) are nowhere encountered in the intima.

The topographic distribution of the lesions of the arterial tree varies from case to case, but, generally speaking, the medium-sized and small arteries are those most frequently affected. The aorta and the pulmonary artery were involved in 5 of the reported cases. It seems worth while to mention that the infants showing involvement of the great vessels were young (see table).

FOOTNOTES TO TABLE

- 1 Durante, G. *Bull et mem Soc anat de Paris* **74** 97, 1899
- 2 Bryant, J. H., and White, W. H. *Guy's Hosp Rep* **55** 17, 1901
- 3 Surbeck, K. *Zentralbl f allg Path u path Anat* **28** 25, 1917
- 4 Verocay, J. *Frankfurt Ztschr f Path* **24** 109, 1920
- 5 Jaffe, R. *Frankfurt Ztschr f Path* **15** 118, 1914
- 6 Hughes, F. W. T., and Perry, C. B. *Bristol Med-Chir J* **46** 219, 1929
- 7 Forrer, H. *Ausgedehnte Gefässverkalkung im frühen Kindesalter*, Inaug Dissert., Zurich, J. H. Meier, 1930
- 8 Iff, M. *Virchows Arch f path Anat* **281** 377, 1931
- 9 Oppenheimer, E. H. *Bull Johns Hopkins Hosp* **63** 261, 1938
- 10 Ross, S. G., and Williams, W. E. *Am J Dis Child* **58** 1142, 1939
- 11 Brown, C. F., and Richter, I. M. *Arch Path* **31** 449, 1941
- 12 Baggenstoss, A. H., and Keith, H. M. *J Pediat* **18** 95, 1941

The vast majority of investigators have enumerated many different sites of sclerotic lesions but have failed to mention the cerebral vessels. The few authors who have described the cerebral arteries have pointed out the absence of arteriosclerosis.

In the present case the aorta and the pulmonary artery, as well as the vessels of the brain, were free of lesions. It seemed of interest to determine the exact point in the vascular tree where the earliest changes would first make their appearance. The common carotid artery provided this opportunity. The proximal end of this artery showed focal change in the intercellular substance of the media, characterized by an increase in its volume and a decrease in the intensity of its staining qualities. A section through the distal end of the same artery revealed more advanced changes, such as fine granular blue-stained deposits formed along the medial surface of the internal elastic membrane (fig 2A). The thyroid and laryngeal arteries, on the other hand, presented extensive plaques of calcium in the media, proliferation of the intima and nearly complete obliteration of their lumens (fig 2B).

As to the clinical signs of the disease under discussion, there are insufficient data available to suggest a symptom complex. In several reported cases, as well as in the present case, failure of general health without obvious cause, persistent anorexia, fretfulness and apathy were the prominent features.

There has been general uncertainty among the authors as to the etiologic factors and the causative mechanism of the disease. However, in an analysis of the cases reported the following facts appear to be significant. Infection, acute or chronic, preceded the death of the infant in 5 reported cases, and was attributed by the authors as the cause of the lesions found.

The theory of an infectious genesis of arteriosclerosis, championed originally by Virchow and supported later, in a modified form, by Klotz¹³ and others, assumes that bacteriotoxins present in the blood injure the intima and prepare it for the subsequent atheromatous change by increasing its permeability. An infectious origin (diphtheria, scarlet fever, measles) of intimal and medial degeneration of the arteries of children was advocated by Wiesel¹⁴. Karsner¹⁵ too has felt that among various factors which operate in the production of arteriosclerosis infectious disease plays a prominent part. However, the etiologic significance of infectious diseases has been disputed by other

13 Klotz, O. *Brit M J* **22** 1767, 1906

14 Wiesel, J. *Wien klin Wchnschr* **56** 1421, 1926

15 Karsner, H. T. *Human Pathology*, Philadelphia, J. B. Lippincott Company, 1942, p. 385



Fig 2—*A*, high power photomicrograph of the common carotid artery, showing early change. Note the incrustation of calcium along the internal elastic membrane. *B*, low power photomicrograph of the thyroid artery.

investigators¹⁶ MacCallum¹⁷ concluded from an analysis of the available clinical, pathologic and experimental evidence that there is little in favor of the idea that acute and chronic infections play a major part in the genesis of arteriosclerosis. As far as the group of infants under discussion is concerned, the authors have pointed out that the earliest lesions detected are in the media and not in the intima as would be expected according to the theory championed by Virchow.

There is no doubt that the arterial calcification observed by Iff in a 1 day old premature infant was acquired in utero. This author insisted that primary arterial damage preceded the calcific change. Furthermore, he maintained that incomplete development of the "ground substance" of the arteries is responsible for the lesions found. This so-called ground substance was much discussed in the German medical literature around 1920. According to Hueck,¹⁸ it is a loose, undifferentiated, syncytium-like tissue, which is the "mother substance" of the arterial wall and a precursor of collagen and elastic tissue and may be found in the arterial wall between the fibers of the latter substances.

Prior to Hueck, Bjorling¹⁹ described loose connective tissue fibers found in the walls of arteries, which he differentiated from collagen and elastic tissue by their metachromatism. Those fibers stained red with Unna's polychrome-methylene blue, in contrast to the blue-staining collagen and elastic fibers. He was unable to demonstrate this substance anywhere in the body aside from the arterial wall. In arteriosclerosis and syphilis of the aorta he observed an increase of this substance in proportion to regression of elastic and muscle fibers. Schultz²⁰ suggested for demonstration of this substance, which he called chromotropic ground substance, cresyl violet (ground substance deep red, muscle fibers pale blue, elastic fibers purple). He was able to demonstrate the chromotropic substance in the alveolar walls of the lungs, in the umbilical cord and the placenta and in the capsule of the glomerulus. According to Schultz, this substance has a special affinity for calcium and lipids, probably because of its high content of chondroitin-sulfuric acid. Ssolowjew,²¹ in a detailed report on the intercellular substance of the arterial wall, stated that it is most abundant in the inner sheaths of the media in arteries of the elastic type. But he was unable to demonstrate it in the walls of the smaller arteries.

16 Zinserling, W. D. Zentralbl. f. allg. Path. u. path. Anat. **24** 627, 1913.
Lange, F. Virchows Arch. f. path. Anat. **248** 463, 1924.

17 MacCallum, W. G. Acute and Chronic Infections as Etiological Factors, in Cowdry, E. V. Arteriosclerosis, New York, The Macmillan Company, 1933, p. 355.

18 Hueck, W. Munchen med. Wchnschr. **67** 535 and 573, 1920.

19 Bjorling, E. Virchows Arch. f. path. Anat. **205** 71, 1911.

20 Schultz, A. Virchows Arch. f. path. Anat. **239** 415, 1922.

21 Ssolowjew, A. Virchows Arch. f. path. Anat. **241** 1, 1923.

Iff expressed the belief that the toxic agent which caused hydramnios in the mother is also responsible for the incomplete maturation of the ground substance in the prematurely born, 1 day old infant, referred to. It is noteworthy that a severe grade of hydramnios was also present in a 2 day old infant with calcification of the pulmonary artery examined by Jaffé⁵

The fact that calcium deposits were found in various organs aside from the arteries led some authors to the assumption that a disturbance in calcium metabolism resulting in inability of the serum to retain all its calcium in solution is responsible for the lesions found.

The present knowledge of calcium metabolism is admittedly incomplete. There is more calcium in the serum at all times than can be accounted for by the laws of simple solution. Barr²² stated that only 25 per cent of the serum calcium can be accounted for by the laws of simple solution. At least two other factors, it is believed, are active to maintain the remainder of the calcium in serum. The more important of these is the hormone of the parathyroid gland, which holds about 50 per cent of the normal serum calcium in solution. In some way the action of vitamin D is related to this fraction of the total serum calcium. Another factor is the combining effect of the serum proteins, which holds about 30 per cent of the normal serum calcium in solution.

It has been shown by Ham and Portuondo²³ in experimental work on animals that abnormal calcifications of tissue may occur with total blood calcium values that are not elevated, and, conversely, the serum calcium can remain high for a long time without producing pathologic calcification.

The serum calcium was not determined in the present case or in the other discussed cases. However, from the clinical data available one may assume that alteration of the blood calcium-phosphorus balance was present in the group of infants showing advanced renal damage (see table).

Since Mitchell²⁴ published his extensive study and thorough review of the literature on renal rickets, this condition has been more frequently reported in the American literature. Mitchell has offered the hypothesis that when there is renal retention of phosphates the waste phosphates are excreted into the intestinal tract, where they combine with the calcium of the food and so block absorption of the latter. The child suffers true calcium starvation, followed by depletion of the calcium of the bones and possibly other tissues. Mitchell reviewed about 190 cases, and in 22 the necropsy notes included the statement that the aorta and other arteries were thickened and sclerotic. An exact mor-

22 Barr, D. P. *Physiol Rev* 12 593, 1932.

23 Ham, A. W., and Portuondo, B. C. *Arch Path* 16 1, 1933.

24 Mitchell. *Am J Dis Child* 40 130, 1930.

phologic description of the lesions was not given. Lightwood²⁵ observed renal dwarfism in a child 2 years and 3 months old. The anatomic character of the arterial lesions in his patient appears identical with that of the lesions found in the group of infants under discussion.

Another condition associated with disturbance of calcium and phosphorus balance and mentioned by several authors as the cause of arterial calcifications is hypervitaminosis D. Observations in man in regard to the arteriosclerotic action of massive doses of vitamin D are relatively scant. Some of the reported cases are of accidental nature, others are of experimental character. The diagnosis of overdosage of vitamin D in the cases reported by Eisler²⁶ and Thomason²⁷ was based solely on abnormal vascular calcification detected by roentgenographic examination. Autopsies were made in the case reported by Putschar²⁸ and 2 cases reported by Thatcher²⁹. In each of these cases extensive calcium deposits were found in the kidney, but none in the arteries. Ross and Williams¹⁰ recorded toxic symptoms from large doses of vitamin D in 4 infants. In this group there were two deaths, one postmortem examination was performed, which revealed widespread calcification of the arteries (inner third of the media) as well as calcium deposits in the kidneys and the myocardium.

Wolf³⁰ attempted to determine the toxic dose of activated vitamin D by administering massive doses to a 4 month old infant afflicted with spina bifida and meningocele, 300,000 U S P units of vitamin D was given daily for two weeks. The patient died following a diagnostic cisternal puncture six weeks after completion of the therapy. Postmortem examination showed deposits of calcium in the renal tubules but none in other organs, including arteries.

Much experimental work has been done to determine the toxic effect of excessive doses of irradiated ergosterol on laboratory animals. It has been shown repeatedly³¹ that massive doses produce medial calcification and intimal proliferation in the walls of the arteries, lesions which appear essentially similar to those found in the group of infants under discussion.

Vanderveer,^{31c} in his report on arteriosclerotic lesions produced in rabbits by excessive doses of vitamin D, pointed out that calcium

25 Lightwood, R. *Arch Dis Childhood* **7** 193, 1932

26 Eisler, F. *Klin Wchnschr* **9** 1846, 1930

27 Thomason, F. *Acta med Scandinav* **93** 505, 1937

28 Putschar, W. *Klin Wchnschr* **8** 858, 1929

29 Thatcher, L. *Edinburgh M J* **38** 456, 1931, *Lancet* **1** 20, 1936

30 Wolf, I. J. *J Pediat* **22** 707, 1943

31 (a) Pallske, G. *Klin Wchnschr* **11** 1060, 1932 (b) Schiff, A. *Virchows Arch f path Anat* **278** 62, 1930 (c) Vanderveer, H. L. *Arch Path* **12** 941, 1931

is first deposited in the intercellular matrix of the media. This author stated "It is surprising what an accumulation of calcaeous material is first deposited over and about the elastic fibers and muscle cells before either show evidence of marked degeneration"

The mechanism by which the calcifications in hypervitaminosis D are produced is still an unsolved problem. Some authors (Vanderveer,^{31c} Wolbach and Bessey³²) have felt definitely that cellular damage occurs first and that calcium is then deposited in the injured areas. Ham,³³ on the other hand, maintained that pathologic calcifications are not preceded by cellular damage, pointing out that he was unable to demonstrate changes in cells twenty-four hours after the drug had been administered to rats and yet after forty-eight hours massive calcification occurred. The possibility has been suggested that vitamin D promotes calcification in the body through a stimulating action on the parathyroid glands. It is notable that the sites at which calcium is deposited in tissues of animals treated with parathyroid extract (Leaner³⁴, Hueper³⁵) are similar to those at which it is deposited in animals as a result of hypervitaminosis D.

McJunkin, Tweedy and Breuhaus³⁶ concluded from their experimental work with parathyroid preparations that the lesions observed in tissues were caused not by direct action of calcium but by a disturbance of the calcium content of the cells altering the calcium balance between these cells and the tissue fluids. Ham³³ suggested that the withdrawal of calcium from the tissue cells during the upswing of the serum calcium curve in hypervitaminosis D should be considered as a possible cause of the injury of the cells.

It may be pointed out, however, that the arterial lesions found in animals treated with massive doses of ergosterol or with parathyroid preparations are similar to those produced experimentally by administration of diphtheria toxin, epinephrine hydrochloride, barium chloride and digitalin. The mode of action of the latter agents is difficult to correlate with calcium metabolism.

Considering the lesions in the patient from the point of view of etiology, one has to admit that the cause is obscure. The healthy appearance of the organs excludes an infectious origin. There is no evidence to indicate an alteration of blood calcium-phosphorus balance, as the parathyroid glands were histologically normal and the kidneys

32 Wolbach, S. B., and Bessey, O. A. *Physiol. Rev.* **22** 233, 1942.

33 Ham, A. W. *Arch. Path.* **14** 614, 1932.

34 Leaner, A. *J. Lab. & Clin. Med.* **14** 921, 1929.

35 Hueper, W. C. *Arch. Path.* **3** 14, 1927, *J. Lab. & Clin. Med.* **19** 1293, 1934.

36 McJunkin, F. A., Tweedy, W. R., and Breuhaus, H. C. *Arch. Path.* **14** 649, 1932.

showed no inflammatory or degenerative changes. The possibility of hypervitaminosis D cannot be entirely excluded. The amount of vitamin D given to the infant, if administered correctly, is in no sense comparable to the doses given experimentally to produce similar lesions in animals.

The fact that the intercellular matrix (ground substance) of the arterial wall was the site of the primary lesions in the clinical group of infants, as well as in experimental animals in which arteriosclerosis was produced by various agents mentioned in a foregoing paragraph, makes one firmly believe that specific changes in the intercellular substance are responsible for the ensuing calcification. One may assume that this intercellular matrix is a system of colloidal nature which is capable of functioning as an organ which can become diseased. Physico-chemical reactions followed by changes in the colloidal state of this substance, or toxic injury of it, may favor formation of calcium deposits. The cause of injury may be the withdrawing of calcium from tissue cells in renal rickets (calcium starvation) or in hypervitaminosis D during the upswing of the serum calcium curve (Ham). Hypercalcemia as such does not deserve the importance ascribed to it, for calcium, as is well known, tends to precipitate in degenerated or nonviable tissue (e g., old tuberculous lesions or infarcts of placenta).

That infectious diseases preceded the deaths of 5 infants cannot be overlooked. The mechanism by which the bacteria or their toxins injure the vascular wall is obscure, as no cellular infiltration occurred in or about the lesions.

The cause of the arterial changes found in the patient remains undetermined.

SUMMARY

Extensive medial calcification of arteries was observed in an infant 10 weeks old. The arterial calcification noted in this and other infants in whom similar findings were made, as shown by a review of the literature, differs from the arteriosclerosis of adults inasmuch as no specific degenerative changes of the intima preceded the medial calcification. The primary site of the calcium deposit appears to be the intercellular substance of the media, which suggests that an injury of this substance is the cause of the calcification.

The arterial lesions found in the aforementioned group of infants resemble the arteriosclerosis experimentally produced in animals by administration of diphtheria toxin, epinephrine hydrochloride, barium chloride, digitalin, parathyroid extract and massive doses of vitamin D.

Mr. E. G. Moore of the Albany Medical College supplied the photomicrographs.

PATHOLOGIC CHANGES RESULTING FROM THE ADMINISTRATION OF STREPTOMYCIN

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AND

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SINCE streptomycin was isolated from *Actinomyces* (*Streptomyces*) *griseus* by Schatz, Bugie and Waksman¹ in 1944, many reports have appeared on the experimental and clinical chemotherapeutic efficacy of this antibiotic agent. The literature concerning this drug has been surveyed recently by Waksman and Schatz²

In view of the definite antibacterial action of streptomycin against numerous gram-negative organisms as well as against certain gram-positive organisms and *Mycobacterium tuberculosis*, the widespread use of this substance is to be anticipated. Studies on the acute lethal toxicity of streptomycin indicate that its toxicity is of a low order³. Preliminary findings on the pathologic effects of streptomycin have been reported from this laboratory as part of a general pharmacologic investigation of the drug (Molitor and co-workers^{3b}). The present communication is an extended report on the pathologic changes produced in laboratory animals by the administration of streptomycin for brief and for prolonged periods.

MATERIALS AND METHODS

Forty-two monkeys, 11 dogs, 350 rats, 100 mice, 10 chickens and 154 guinea pigs were used in these experiments. All these animal species were housed in

From the departments of pathology of the Merck Institute for Therapeutic Research, Rahway, N J, and the Newark City Hospital, Newark, N J, and the Office of the Chief Medical Examiner, Essex County, N J.

The work described in this paper was done in part under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development of the National Research Council and the Merck Institute for Therapeutic Research, Rahway, N J.

1 Schatz, A, Bugie, E, and Waksman, S A. *Proc Soc Exper Biol & Med* 55 66, 1944

2 Waksman, S A, and Schatz, A. *J Am Pharm A (Sci Ed)* 34 273, 1945

3 (a) Robinson, H J, Smith, D G, and Graessle, O E. *Proc Soc Exper Biol & Med* 57 226, 1944. (b) Molitor, H, Graessle, O E, Kuna, S, Mushett, C W, and Silber, R H. *J Pharmacol & Exper Therap* 86 151, 1945

air-conditioned quarters at a temperature of 75 F and a relative humidity of 50 per cent. The monkeys, *Macacus rhesus*, weighing between 3 and 6 Kg, were fed a mixed diet consisting of Purina dog chow, fresh fruits and vegetables. The dogs, mongrels weighing 8 to 11 Kg, were given a mixture of Gaines dog meal, fresh horse meat and milk. Nutritionally complete diets were also provided for the rats (Wistar strain), mice (CFW strain), chickens and guinea pigs.

Most of the studies were carried out with streptomycin hydrochloride concentrates⁴ varying in potency from 250 to 400 micrograms of streptomycin base per milligram of solids⁵. Highly purified streptomycin, with a potency of 800 micrograms streptomycin base per milligram of solids, was used in only a few experiments. The streptomycin was administered subcutaneously, intramuscularly or intravenously in an aqueous neutral solution in doses of 10 to 200 mg per kilogram. Many of the animals received the drug daily for periods of about five days, but in several experiments the dosing period was extended to several months or more.

Samples of urine were collected alternately with and without toluene. The erythrocyte, leukocyte, differential and platelet counts, the sedimentation rate, the hematocrit value, the icteric index and the prothrombin level (on both 100 and 125 per cent plasma) were determined on blood samples drawn at frequent intervals. Tissues for microscopic examination were fixed in neutral solution of formaldehyde USP (1:10) and also in one or more of the following: Helly's fluid,⁶ formaldehyde-alcohol (4 per cent formaldehyde in absolute ethyl alcohol), absolute acetone and Regaud's solution. The stains employed were hematoxylin-eosin, sudan IV, Best's glycogen stain and others as indicated. Since the most complete studies were made in monkeys and dogs, the major portion of this communication will deal with these species.

PATHOLOGIC EFFECTS ON THE LIVER AND THE KIDNEY

Macroscopic Changes—The internal organs of most of the species treated with streptomycin appeared normal, but pale areas were noted in the livers and the kidneys of dogs and monkeys. The median lobe of the liver of 1 dog showed tan areas suggestive of necrosis. Occasionally in dogs the gallbladder was distended, the lower part of the jejunum and the ileum were bile stained and the stools appeared green and tarry.

4 All streptomycin used in this study was supplied by the Research Laboratories of Merck & Co., Inc.

5 The potency of a streptomycin concentrate was formerly expressed in terms of units, one unit being that quantity which will just inhibit a given strain of *Escherichia coli* in 1 cc of nutrient medium. As recommended by the Food and Drug Administration, the potency of streptomycin is now designated in terms of its equivalent in weight of pure streptomycin base. One microgram of streptomycin base is equivalent to 1 unit. In this paper all doses are expressed in terms of weight of streptomycin base administered, the weight does not refer to the actual weight of the streptomycin concentrate.

6 Potassium dichromate, 2.5 Gm, mercuric chloride, 5 Gm, sodium sulfate, 1 Gm, water, 100 cc, and solution of formaldehyde, USP, 5 cc, added before use to each 100 cc of fluid. This is a modified form of Zenker's fluid.

Microscopic Changes—(a) Liver The cytoplasm of the hepatic cells appeared vacuolated and sparsely granular in dogs and monkeys treated with streptomycin in doses of 25 mg per kilogram or more. Sudan IV stain revealed moderate to marked fatty metamorphosis, with the fat present in the cord cells or the Kupffer cells in fine to moderate-sized globules (*A*, fig 1). When present in large quantities the fat showed uniform distribution throughout the lobules (*B*, fig 1), pericentral concentration was noted when a lesser amount was present. Glycogen was equivalent to or greater than that found in control animals. Like the fat, its lobular distribution was uniform (*C*, fig 1). No inverse relationship existed between the amount of fat and the amount of glycogen present. In fact, some of the livers containing the greatest quantity of glycogen also showed the most pronounced fatty metamorphosis.

Multiple small foci of necrosis, with round cell and occasionally polymorphonuclear cell infiltration (*A*, fig 2), were seen in the livers of a few dogs. Fibroblastic proliferation was seen also in some areas. Fine yellow granules were present in the cord cells, particularly around the bile canaliculi. Iron-positive pigment deposits were seen in the centrilobular areas.

In an experiment designed to permit study of the genesis and the course of the fatty metamorphosis in the liver, monkeys were given five daily intravenous injections of 25 mg per kilogram and killed in pairs at intervals of one, ten, thirty and sixty days after the last injection. A small amount of lipid material was present in liver sections after one day. On the tenth day an increased amount was present (*B*, fig 1). Little fat was seen after thirty days (*D*, fig 1), and by sixty days it was absent. These data indicate that the fatty metamorphosis resulting from the administration of streptomycin is a reversible phenomenon and does not represent progressive degeneration.

Monkeys treated by daily subcutaneous injection of 25 mg per kilogram for sixty-six consecutive days did not present pathologic changes any more impressive than those observed in monkeys given the same dose of the drug intravenously for a period of only five days.

The fatty change occurred in animals treated with highly purified streptomycin (800 micrograms streptomycin base per milligram of solids) as well as with less pure concentrates.

No gross or microscopic changes which could be attributed to streptomycin were observed in the livers of rats (100 mg per kilogram daily, subcutaneously, for seventy-two days), guinea pigs (60 mg per kilogram daily, subcutaneously, for seventy days), mice (200 mg per kilogram daily subcutaneously for ten days) or chickens (100 mg per kilogram daily subcutaneously, for fourteen days).

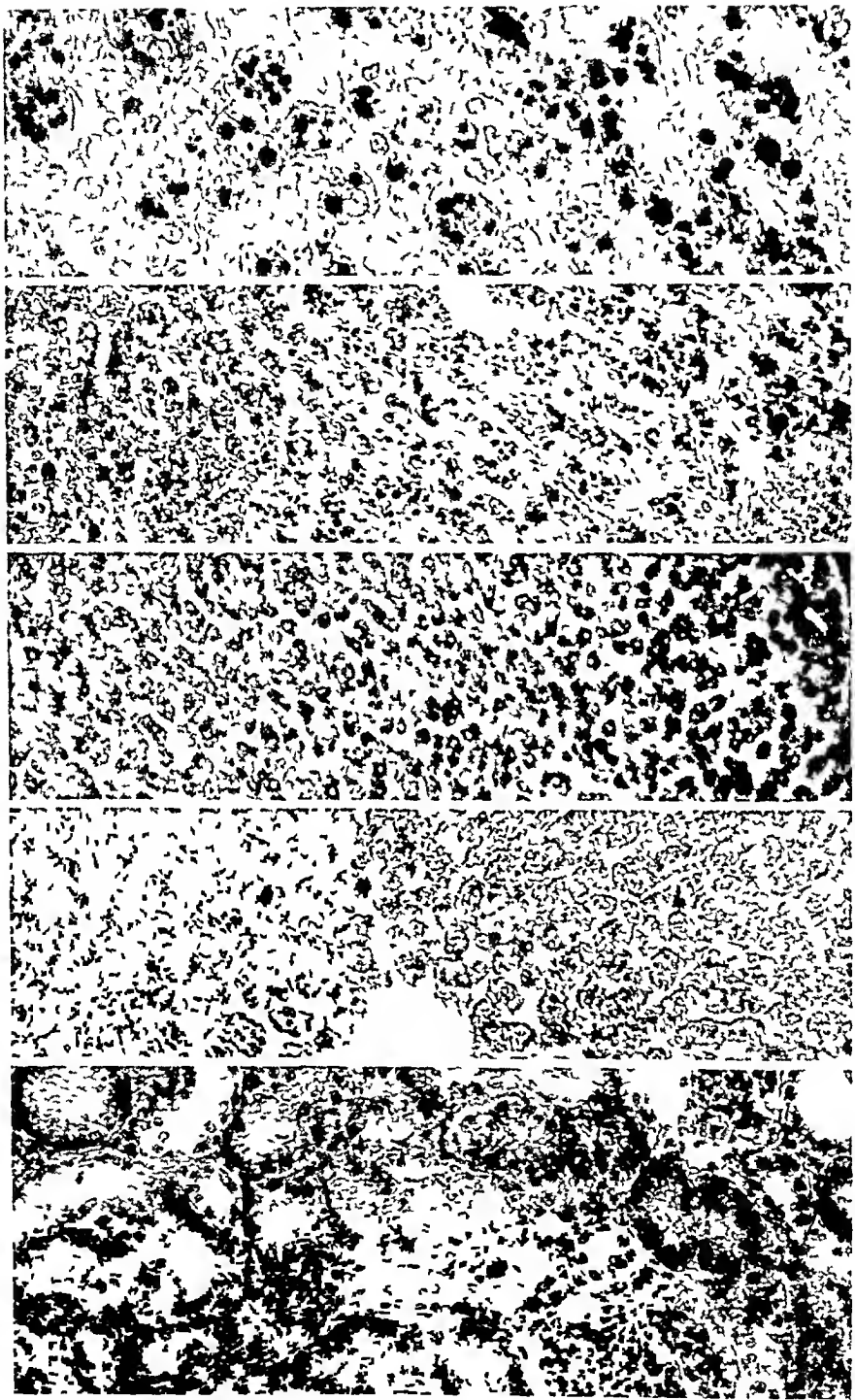


Figure 1
(See legend on opposite page)

(b) Kidneys Examination was made of the sediment of centrifuged urine of monkeys, dogs and rats. In the urine from a few monkeys given several large doses of streptomycin or smaller doses for prolonged periods, an occasional cast, epithelial cell or blood cell was seen, but the presence of these formed elements in the urine was never consistent or great in extent and can be considered to be of little significance, particularly since the urine of control animals at times showed a similar picture. The urine of rats treated by parenteral injection of 100 mg per kilogram for approximately two and one-half months showed no changes. That of dogs receiving 50 or 100 mg per kilogram daily showed, within a few weeks, casts, epithelial cells and leukocytes. An occasional erythrocyte was seen in the urine of a few animals. We have reported the occurrence of albuminuria in several of these animals^{3b}. A report of the biochemical studies conducted on these animals will be published elsewhere.⁷

On histologic examination, pale eosinophilic granular detritus was present in the glomerular spaces and the tubules of the kidneys of several monkeys in which proteinuria had developed after treatment for five to ten days with 100 or 200 mg per kilogram. No casts, however, were present in the tubules. Several kidneys had a slight degree of fatty degeneration in the convoluted tubules (*E*, fig 1). In the kidney, as in the liver the fatty metamorphosis was found to be reversible. Thus in monkeys killed ten days after the last of five daily intravenous injections of 25 mg per kilogram fat was present in the renal parenchyma, but none was seen in animals killed thirty or sixty days after such treatment. The fine lipid globules occupied the basal portion of the epithelial cells or the interstitial tissue of the collecting tubules. Rarely, brownish refractile granules were present also in the epithelium.

7 Silber, R. H., Porter, C., and Clark, I. To be published

EXPLANATION OF FIGURE 1

Pathologic changes in tissues of monkeys treated by intravenous injections of streptomycin—25 mg per kilogram daily for five days

A, fatty metamorphosis of liver. Lipoid material is present in fine to moderately large globules. The animal was killed ten days after the last injection. Sudan IV stain, $\times 300$.

B, lipoid material uniformly distributed throughout a hepatic lobule. Low power magnification of *A* ($\times 130$).

C, liver containing abundant, evenly disposed glycogen deposits. The animal was killed ten days after the last injection. Best's carmine stain, $\times 130$.

D, liver of a monkey used in an experiment to demonstrate reversibility of fatty metamorphosis. Only a small amount of lipoid material is present in the pericentral area. The animal was killed thirty days after the last injection. Sudan IV stain, $\times 130$.

E, fatty metamorphosis of kidney. Tubules containing lipoid globules are present between the two glomeruli and in the lower portion. The animal was killed ten days after the last injection. Sudan IV stain, $\times 130$.

of the convoluted tubules. Staining of renal tissue for glycogen revealed none, and examination of unstained frozen sections with Nicol prisms failed to reveal doubly refractile droplets of cholesterol esters.

Focal necrosis (*B*, fig 2), sometimes accompanied by epithelial desquamation, was observed in the convoluted tubules of the kidneys of 1 dog, and patchy areas of round cell infiltration were seen in the renal

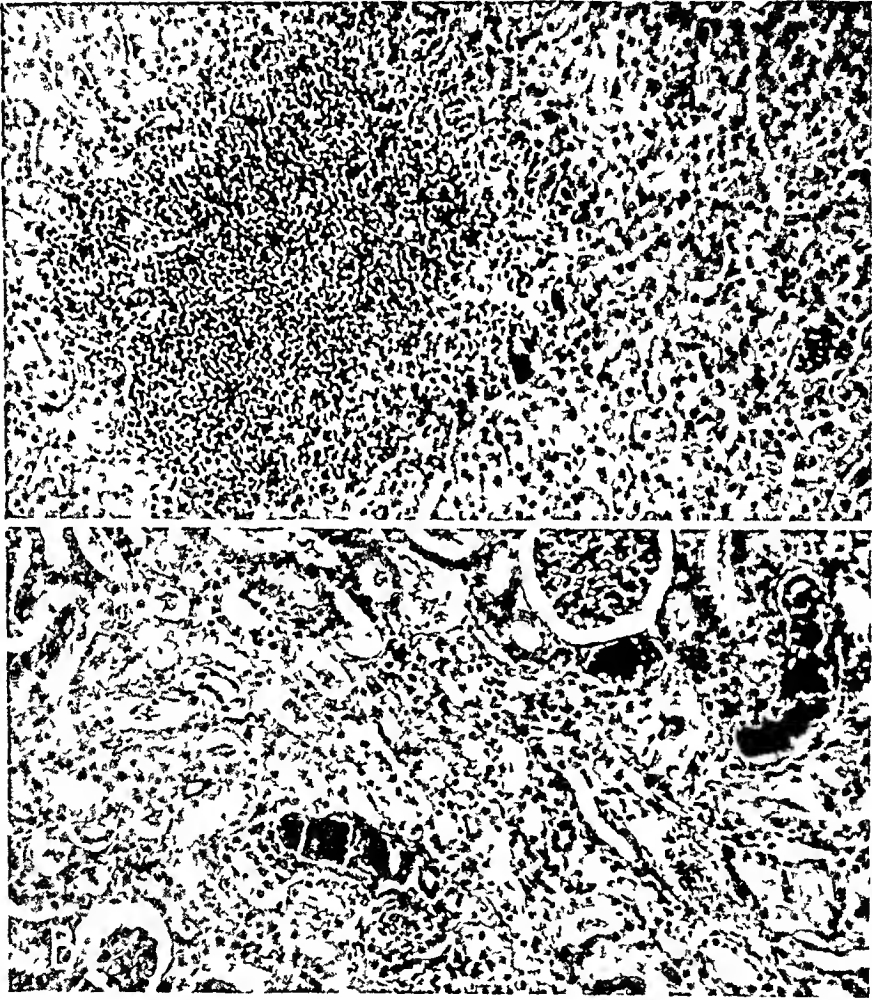


Fig 2—Pathologic changes in tissues of dogs treated by intramuscular injection of streptomycin—100 mg per kilogram daily for twenty consecutive days. The animals were killed fifteen days after the last injection.

A, focal necrosis of liver. Note also small aggregations of lymphocytes. Hematoxylin and eosin, $\times 150$.

B, focal necrosis with calcification in the convoluted tubules of the kidney. The glomeruli appear normal. Hematoxylin and eosin, $\times 150$.

tissue of 2 dogs treated by subcutaneous injection of 100 mg per kilogram per day for twenty days. Basophilic granular material (calcium) was frequently present at the necrotic sites. Hyaline casts and

occasionally iron-positive casts occurred in the collecting tubules. Much stainable lipid material was evident in the cells of the convoluted segment of the nephron and the limbs of Henle's loop, less was seen in the epithelium of the collecting tubules.

The presence of granular albuminous detritus in the glomerular spaces and tubules of the kidneys of some monkeys treated with streptomycin indicated increased permeability of the glomerular capillary membranes. That this change is a reversible one is suggested by the fact that certain monkeys which had shown clinical evidence of proteinuria and later recovered did not have granular detritus present in the nephrons.

None of the other species given injections of streptomycin showed pathologic changes in the kidneys.

TOXIC EFFECTS ON THE NERVOUS SYSTEM

Two dogs which had received daily subcutaneous injections of 100 mg per kilogram of a streptomycin concentrate (potency, 250 mg streptomycin base per milligram of solids) for twenty days were killed fifteen days after the last injection. Both animals had shown disturbances of equilibrium during the latter part of the dosing period. They were disposed to walk with a wavering gait in wide circles, with the head kept to one side. An impairment of auditory acuity was suggested by a failure of the dogs to respond normally to an unexpected loud sound. At the time of autopsy, several weeks after this condition was first observed, no improvement was noted in either animal. One of 3 dogs treated with 50 mg per kilogram exhibited disturbances of equilibrium and of gait similar to, but milder than, those seen in the animals which had received 100 mg per kilogram. The symptoms appeared after one and one-half months of treatment, but despite continued treatment this animal recovered several weeks later. Two dogs treated by subcutaneous injection for twenty days with samples of streptomycin of relatively high potency (640 to 710 micrograms of streptomycin base per milligram of solids) at a dose level of 100 mg per kilogram failed to show neurotoxic symptoms.

In view of the equilibrial and possible auditory disturbances found in some of these dogs, histologic studies were made on the cerebrum, the cerebellum and the medulla and also on the acoustic, brachial and sciatic nerves. No pathologic alterations were observed in these tissues stained with hematoxylin-eosin, Bodian's stain and the Pal-Weigert stain.

It is believed therefore, that the toxic manifestations exhibited by these animals may be due to degenerative changes in the vestibular and auditory end organs. To elucidate this possibility histologic studies

of temporal bones are being conducted in collaboration with Dr E P Fowler Jr.⁸ It is also possible that there may be some direct effect on the cerebellum or other nervous tissues which will require further study by more extensive neuropathologic methods

EFFECTS ON THE BLOOD AND THE BLOOD-FORMING ORGANS

Complete and frequently determined blood values revealed as the only significant change slight normocytic anemia. This was usually observed after several daily injections of 50 mg per kilogram or more in the monkey or after one week of daily injections of 100 mg per kilogram in dogs. The anemia was transient, the cell counts returning to normal shortly after withholding of the drug. It persisted in 2 dogs during a period of reduced intake of food. Occasionally a slight increase in erythrocyte sedimentation rate occurred, due probably to the state of anemia. No significant changes in total or in differential leukocyte counts, platelet counts, prothrombin levels or icteric indexes were observed at any time.

Slight anemia was seen in rats receiving subcutaneous injections at a dose level of 100 mg per kilogram of a low potency streptomycin concentrate (60 micrograms of streptomycin base per milligram of solids). When this was followed with samples of higher potency, the red cell count and the hemoglobin level returned to normal, and no further changes were observed in the blood picture, although treatment was continued for a total of seventy-two days.

Histologic examination of the femoral marrow of these monkeys, dogs and rats revealed no pertinent changes. In some of the animals the spleen showed an increased amount of hemosiderin, but the structure was normal.

The occurrence of anemia following streptomycin therapy would not appear to be of serious consequence, since the anemia is transient and no involvement of the marrow is indicated. In the clinical investigation of streptomycin, Zintel and co-workers,⁹ Heilman and co-workers¹⁰ and Foshay¹¹ observed no significant changes in the blood of their patients.

LOCAL EFFECTS AT SITES OF ADMINISTRATION

Muscle and Skin—At autopsy the most striking gross effects noted were lesions occurring at the sites of injection. Small areas of

8 Fowler, E P, Jr, department of otolaryngology, College of Physicians and Surgeons, Columbia University, New York

9 Zintel, H A, Flippin, H F, Nichols, A C, Wiley, M M, and Rhoads, J E. *Am J M Sc* **210** 421, 1945

10 Heilman, D H, Heilman, F R, Hinshaw, H C, Nichols, D R, and Herrell, W E. *Am J M Sc* **210** 578, 1945

11 Foshay, L. *J A M A* **130** 393, 1946

necrosis were seen in muscles in which as little as 10 mg per kilogram had been injected. Dry scabs and occasionally ulcers of the epidermis were present in many animals treated by subcutaneous injection. The degree of local damage showed a direct relation to the potency of the material used. Thus, at a level of 100 mg per kilogram no apparent effect resulted from samples with a potency of 225 to 400 micrograms of streptomycin base per milligram of solids, whereas a low potency preparation (30 micrograms per milligram) was responsible for the production of many open ulcers and scabs. Only a few small dry scabs were seen in the monkeys which received streptomycin of intermediate potency (135 micrograms per milligram).

Vein—One monkey, weighing 4 Kg, was given by continuous intravenous infusion a total of 1.9 Gm of streptomycin over a forty-two hour period. Microscopic examination of tissue from the cannulated area revealed an organizing thrombus in the vein and panphlebitis. Localized hemorrhage was also evident.

Pleural Cavities—In anticipation of the use of streptomycin in the clinical therapy of empyema, experiments were conducted to determine the local effects produced by intrapleural injections of the drug. Solutions containing 1, 10 or 100 mg of streptomycin per cubic centimeter were injected into the pleural cavities of rabbits at a dose of 1 cc per kilogram. Two animals were used at each dose level. Four days after injection all animals had localized congestion and effusion of pleural fluid. Those which received the 100 mg dose had small areas of hemorrhage in the pleural wall and fibrinous adhesions between the lung, the diaphragm and the pleural wall.

Gastrointestinal Tract—Since streptomycin is absorbed to only a slight extent on oral administration,¹² one would not expect to find in the viscera of animals so treated pathologic changes comparable to those observed after parenteral dosage. Necropsy of mice dead from an oral dose of the drug revealed an excessive amount of fluid in the gastrointestinal tract and numerous small hemorrhagic lesions along the intestine. Similar findings were recorded when sodium chloride solutions of approximately the same osmotic pressure as the solutions of streptomycin were given. As suggested in a previous report,^{3b} it appears that these effects on the gastrointestinal tract are due primarily to the osmotic concentrations of the streptomycin solutions employed, although the drug itself may have exerted a local irritating action in addition.

12 Reimann, H. A., Elias, W. F., and Price, A. H. *J. A. M. A.* **128** 175, 1945. Stebbins, R. B., Graessle, O. E., and Robinson, H. J. *Proc. Soc. Exper. Biol. & Med.* **60** 68, 1945.

Mice which received streptomycin in their diet (25 mg per gram of diet) for a period of four months showed no pertinent gross or microscopic alterations in their tissues

CHANGES IN OTHER ORGANS

Buccal Lesions—In 1 monkey given five daily subcutaneous injections of 100 mg per kilogram a small suppurative ulcer developed at the tip of the tongue. This lesion was similar to but much less severe than those produced in both monkeys and dogs by streptomycin.¹³

Adrenal Glands—The adrenal gland of 1 monkey which had received a low potency preparation contained a calcified mass at the junction of cortex and medulla. This isolated observation may be of no significance.

Other Viscera—No significant pathologic changes were seen in the following organs of any of the animals treated parenterally with streptomycin: heart, aorta, lungs, spleen, lymph nodes, pancreas, thyroid glands, adrenal glands, pituitary gland, testes, prostate, bladder, gastrointestinal tract, eyes.

COMMENT

Dogs and monkeys treated with streptomycin concentrates were found to have fatty metamorphosis of the liver and less often of the kidneys. Focal necrosis was seen infrequently in these organs. Long-continued dosage with streptomycin did not result in pathologic changes which were more marked than those seen after shorter periods of treatment. Evidence of pathologic damage in man following therapy with streptomycin is lacking.

However, neurotoxic signs similar to those observed in dogs in the present experiments have been seen in man. Thus Hinshaw and Feldman¹⁴ described transient deafness and disturbances of the vestibular apparatus with marked vertigo in patients treated with large doses of streptomycin for prolonged periods. They are inclined to attribute this neurotoxic effect to involvement of the eighth nerve. More recently Lawrence¹⁵ has observed cerebellar ataxia without apparent involvement of the eighth nerve or the labyrinth in a patient given a total dose of 14,000,000 units (14 Gm) of streptomycin. The patient recovered within six weeks. No serious or uncontrollable toxic effects were encountered by Herrell and Nichols¹⁶ after short term use of strepto-

13 Mushett, C. W., and Martland, H. S. *Federation Proc.* **5**: 194, 1946.

14 Hinshaw, H. C., and Feldman, W. H. *Proc. Staff Meet., Mayo Clin.* **20**: 313, 1945.

15 Lawrence, E. A. Personal communication to the authors.

16 Herrell, W. E., and Nichols, D. R. *Proc. Staff Meet., Mayo Clin.* **20**: 449, 1945.

mycin in 45 patients. In limited studies of affected dogs no lesions have been observed in the eighth nerve or in the brain. Studies on the vestibular and auditory end organs may reveal pertinent information on this matter.

The degree of local tissue damage following the parenteral administration of streptomycin concentrates showed a direct relation to the potency (purity) of the material injected. Whereas a low potency sample produced marked damage, samples of higher potency were without injurious effect. The more purified samples of streptomycin are virtually free of the toxic component which, in earlier samples of lesser purity, was responsible for a histamine-like action.¹⁷

Since the local damage and histamine-like action of certain streptomycin concentrates can be ascribed to impurities, there remains the possibility that the hepatotoxic, renotoxic and neurotoxic effects seen in animals treated with streptomycin may also be due to impurities. Further studies with chemically pure streptomycin will serve to elucidate this possibility.

SUMMARY

The parenteral administration of highly purified as well as average streptomycin samples resulted in fatty metamorphosis of the liver in monkeys and dogs. Large doses of streptomycin concentrates produced small foci of necrosis in the livers of a few dogs.

Fatty metamorphosis was observed less often in the kidneys of monkeys and dogs treated with streptomycin. Albuminous detritus appeared in the subcapsular spaces and the tubules of the kidney in some of the monkeys. Hyaline and granular casts, epithelial cells and occasionally blood cells were seen in the sediment of the centrifuged urine of dogs receiving large doses of streptomycin concentrates. Focal tubular necrosis occurred in the kidneys of one dog.

The fatty change observed in the liver and the kidney was found to be reversible. It was not followed by permanent pathologic damage.

Complete studies of the blood of monkeys, dogs and rats treated by injection of streptomycin revealed as the only significant change slight normocytic anemia which disappeared on cessation of dosage.

Prolonged administration of streptomycin concentrates of average purity (230 to 310 micrograms of streptomycin base per milligram of solids) resulted in neurotoxic effects in dogs, manifested by disturbances of equilibrium and possibly of auditory acuity. Spontaneous recovery occurred in 1 animal. No lesions which could explain these effects were evident in the limited material studied. Streptomycin samples of higher potency (640 to 710 micrograms of streptomycin base per milligram of solids) did not produce neurotoxic symptoms in dogs.

17 Molitor and others^{3b} Herrell and Nichols¹⁶

Case Reports

PRIMARY PULMONARY VASCULAR SCLEROSIS

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THE CONDITION to be described is one which seems to us to fit into primary pulmonary vascular sclerosis as defined by Brenner¹. The requirements listed by Brill and Krygier² in their recent review of the subject are (1) significant hypertrophy of the right ventricle but not of the left and (2) absence of all factors commonly believed to cause secondary vascular sclerosis or pulmonary hypertension, such as mitral stenosis or chronic pulmonary disease.

REPORT OF A CASE

A married woman 56 years of age entered the Robert Breck Brigham Hospital, Nov. 24, 1942, because of dyspnea and pain in the lower part of the chest and the back. Since her climacterium, at the age of 37, she had become increasingly irritable emotionally. She also had noted increasingly severe symptoms of what was diagnosed as Raynaud's disease by a number of physicians whom she consulted. During the summer and fall prior to her admission she had become breathless and on exertion had noted pain across the lower part of the chest and the back. She had been constantly tired and had had a nonproductive cough.

Her mother died at the age of 64 from a "shock" as did her father at the age of 54. The patient thought that her mother had had symptoms similar to her own.

The patient was a well developed, moderately obese white woman (height, 162.5 cm, weight, 65.9 Kg at autopsy). She was dyspneic and cyanotic. Her hands and feet showed marked cyanosis. Slight stimuli, such as touching the feet or the hands with a cold object, produced pallor. The skin of the backs of the hands, the forearms and the soft parts of the hands and fingers seemed to be coarse and thickened. The skin of the feet and toes was similar. There was no clubbing of the fingers or the toes. There was moderate edema of the lower extremities. The heart seemed to be enlarged to the right, but there were no murmurs. Expansion of the lungs was good. There were some rales at both bases. The liver was palpable.

The patient's red blood cell count was 5,170,000. Her hemoglobin concentration was 14.2 Gm per hundred cubic centimeters of blood. The white blood cell count, the urine and the results of the routine blood serum studies were not remarkable. Roentgenograms taken on November 27 showed an enlarged heart with fulness over the pulmonary conus. Electrocardiograms showed marked right ventricular preponderance.

From the Mallory Institute of Pathology, Boston City Hospital, and the Robert Breck Brigham Hospital.

1 Brenner, O. Arch Int Med 56: 211, 457, 724, 976 and 1189, 1935. (See section on primary pulmonary vascular sclerosis, pp. 976-990.)

2 Brill, I. C., and Krygier, J. J. Arch Int Med 68: 560, 1941.

The patient improved for a while on rest in bed. A week prior to death her left leg became swollen and tender. She was thought to have acute phlebitis. The patient continued to be more and more dyspneic and cyanotic until her death on December 24.

Autopsy (thirteen hours post mortem).—The body was that of a well developed, moderately obese white woman. There was moderate pitting edema of the feet, the legs and the thighs, more marked on the left. The tips of the fingers were somewhat blue, and the overlying skin appeared to be atrophic. There was no clubbing of the fingers.

There was 500 cc of clear yellow fluid in the right pleural cavity, the left was dry.

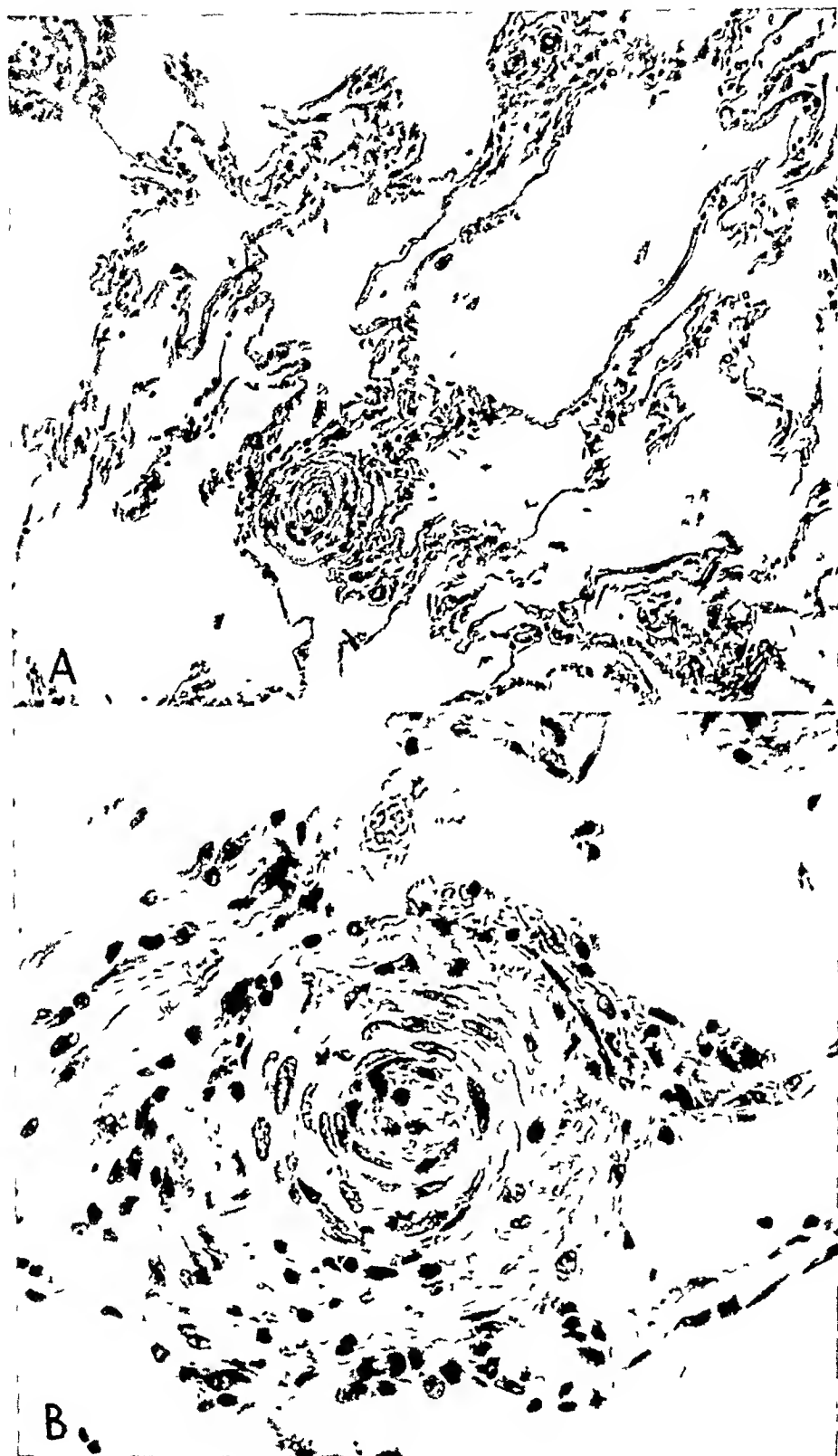
The heart weighed 280 Gm. It was not remarkable except for marked hypertrophy of the right ventricle, which measured 0.5 to 0.7 cm in thickness. The right ventricle and auricle were somewhat dilated, and the right auricle was moderately hypertrophied. The right auricular appendage was filled with an antemortem thrombus which showed piriform softening.

The right lung weighed 430 Gm, the left, 270 Gm. The branch of the pulmonary artery to the right lung was completely filled with thrombi of varying ages. The most recent was attached to the arterial wall and not readily torn free. The lungs were dry and crepitant throughout except for a wedge-shaped area of consolidation which extended from the hilus of the right lung to involve most of the diaphragmatic surface of the lower lobe.

The spleen weighed 120 Gm and was acutely congested. The gastrointestinal tract was not abnormal except for a small rectal polyp. The liver weighed 1,220 Gm. The cut surface oozed considerable dark blood and revealed dark red central lobular zones, which were slightly depressed. The kidneys, the adrenal glands and the genital organs were not remarkable except for congestion. The left iliac vein was found obstructed with an antemortem thrombus, which was not firmly attached to the vessel wall.

Microscopic Examination.—The myocardium, the epicardium and the endocardium were not abnormal. The right auricle contained an antemortem thrombus, which showed early peripheral organization. The spleen, the liver and the kidneys were markedly congested. There was some central hemorrhagic necrosis of liver cells. The skin of the right forefinger was within normal limits.

The pathologic changes of interest were in the lungs. Sections of the lower lobe of the right lung revealed an area in which the alveoli were filled with red cells and in which there was infarct necrosis of the alveolar walls. The histologic picture in multiple sections taken from the remainder of the right lung and from the left lung was essentially the same. There was moderate emphysema, as shown by dilated, thin-walled alveoli. There was no thickening of the interalveolar septums. The main pathologic development in the lungs was a marked proliferation of the endothelium of the majority of the arterioles. This proliferation was of the type seen in rapidly progressive nephrosclerosis. The endothelial cells were arranged in concentric rings, which resulted in the narrowing of the lumens of the arterioles, occasionally to the point of apparent obliteration. However, no areas of necrosis were noted in the arteriolar walls. Sections through the larger branches of the pulmonary arteries showed moderate atheromatous changes in the intima. There was no evidence of left-sided heart failure in any of the sections of lung that were studied. All alveoli were well aerated and contained no edema fluid or "heart failure" cells. The vessels in the alveolar walls were not congested.



A shows the moderate alveolar dilatation observed in the lung and three small vessels with proliferative changes Phloxine and methylene blue, $\times 100$

B shows the detail of the proliferative changes in an arteriole of the lung Phloxine and methylene blue, $\times 450$

Elastic tissue stains revealed no alteration in the elastic fibers. Stains with the Lee-Brown modification of Mallory's aniline blue collagen stain did not reveal any unusual changes in the alveolar walls.

Permission to report this case was given us by Dr. Burton F. Hamilton, who also supplied the summary of the clinical record.

COMMENT

Clinically it was thought that the patient had a peripheral vascular disease (Raynaud's disease) which had also affected the pulmonary circulation with resultant dilatation of the heart (cor pulmonale) and failure of the right side of the heart. Five cases of this sort have been reported recently,³ in one of which an autopsy was performed. The microscopic alteration of the lungs in the last-mentioned case consisted of marked fibrosis of the alveolar walls with minimal involvement of the vessels, the heart was moderately enlarged but did not show cor pulmonale.

In the present case the observations were not at all similar. There was no evidence of pathologic change in the digital skin examined, and the findings of interest were confined to the heart and the pulmonic vascular tree rather than to the alveolar walls. There was moderate atherosclerosis of the larger arteries with marked proliferative arteriosclerosis and moderate right ventricular hypertrophy. There were no vascular changes similar to those seen in the lung. Although there was moderate emphysema in the other organs, we believe that the pulmonary hypertensive vascular disease in this case cannot be explained on that basis.

As is the case in peripheral arterial hypertension and arteriosclerosis—more especially that of the rapidly progressive type—pulmonary arteriolar and arterial hypertension undoubtedly precedes arteriolar and arterial changes, which may therefore be considered resultant rather than causative. The cause of pulmonary hypertensive vascular disease is unknown at present as is the cause of so-called essential hypertension. As Brenner¹ stated

it seems unlikely that the hypertrophy of the right ventricle and the heart failure are directly due to the lesions in the pulmonary vessels, since similar symptoms and hypertrophy of the right heart may occur without pulmonary vascular lesions, and lesions greater than those in many of the cases that have been reported may occur without hypertrophy of the right ventricle or symptoms of heart failure.

It is possible that the pulmonary vascular lesions and the ventricular hypertrophy and failure are due to some unknown common cause rather than that they are related as cause and effect.

³ Linenthal, H., and Talkov, R. *New England J. Med.* **224**: 682, 1941, 227-433, 1942.

Pathologically, primary pulmonary vascular sclerosis is not as clear-cut a picture as might be expected from the rather dogmatic criteria cited in the introduction. As emphasized by Brenner, the lesions are varied. Lesions of the intima of the larger arteries predominate in some cases, lesions of the endothelial lining of the smaller arteries and arterioles, in others. In some cases the lesions are localized in one portion of the lung. In others the lesion seems to be one primarily of the elastica and the media.

Thus, as suggested by Brill and Krygier, this problem is not at present susceptible to explanation by histologic methods. Perhaps, as they also suggested, when a means of determining the tension in the pulmonary circuit is developed, one may be many steps closer to the solution of this problem.

SUMMARY

In the case of primary pulmonary vascular sclerosis described, the most important pathologic change was proliferative pulmonary arteriole-sclerosis, which probably resulted from hypertensive pulmonary vascular disease.

PRIMARY ADENOCARCINOMA OF THE UMBILICUS

GERARD DESFORGES, M D, BOSTON

ACCORDING to Cullen,¹ cancer of the umbilicus, whether primary or secondary, is exceptionally rare. This fact is borne out by the finding of only 9 such tumors in 18,668 autopsies and 123,825 surgical specimens seen at the Mallory Institute of Pathology of the Boston City Hospital.

Tumors of the umbilicus are classified by Cullen¹ as follows

A Primary umbilical carcinoma

- 1 Squamous cell carcinoma
- 2 Adenocarcinoma

B Secondary umbilical carcinoma

- 1 From the stomach
- 2 From the gallbladder
- 3 From the intestine
- 4 From the ovaries
- 5 From the uterus
- 6 From other abdominal organs

Perhaps owing to the rarity of its occurrence, the possibility of adenocarcinoma primary in the umbilicus is often overlooked, particularly if signs or symptoms suggestive of visceral tumor are present, leading to the conclusion that the umbilical lesion is secondary.

The case to be reported is an instance of a primary umbilical lesion in which, in the presence of intestinal symptoms, a biopsy report of adenocarcinoma led to the erroneous belief that metastatic disease was present. Hence, surgical removal was not attempted.

A short review of the embryology and the anatomy of the umbilical region will not only show the possible origin of adenomatous lesions but indicate why a primary tumor of the umbilicus is so rare.

As the embryo enlarges, its ventral unclosed area becomes relatively smaller. This region at the junction of the embryonic and the extra-embryonic territory is the primitive umbilicus.² Passing through this primitive structure are the omphalomesenteric or vitelline duct, the allantois and the accompanying blood supply. The allantois is a true vestigial structure and quickly disappears only to leave a few fibrous strands connecting with the urachus at the umbilicus. Anomalous development of these structures leads to such well known umbilical

From the Mallory Institute of Pathology, Boston City Hospital

1 Cullen, T. S. *The Umbilicus and Its Diseases*, Philadelphia, W. B. Saunders Company, 1916, pp. 400-448.

2 Arey, L. B. *Developmental Anatomy*, Philadelphia, W. B. Saunders Company, 1942, p. 108.

defects as fecal fistula (persistent patency of the omphalomesenteric duct) and to urinary fistula (persistently patent urachus)

Histologically, glandular epithelium may be observed in both patent urachi and persistent omphalomesenteric ducts³ This observation may well be expected when it is realized that the omphalomesenteric duct is directly connected with the primitive midgut and that the early hindgut possesses the anlage of both the rectum and the urachus As for the urachal remnant, its epithelium does not become specialized but retains the primitive cell's potentiality of differentiating into any epithelial cell type⁴

Normally these fetal structures proceed to total obliteration, and the umbilicus becomes a small circular pad of dense connective tissue covered by thin squamous epithelium It is devoid of hair follicles, sweat glands and sebaceous glands Therefore, save for the rare remnants of omphalomesenteric duct and urachus which may persist, no structures exist from which adenocarcinoma may arise

REPORT OF A CASE

A 79 year old single white woman entered the Boston City Hospital, April 11, 1945, with the chief complaints of weakness, loss of weight, swelling of the abdomen and some abdominal pain present during the last four months

About one year before entry she noticed discoloration of the umbilicus, with pruritus This subsided after two months During the whole year, however, she had increasing constipation and pain in the back which radiated down the legs and was worse at night Five weeks before entry discoloration and pruritus of the umbilicus reappeared, and now there was also a malodorous yellowish discharge Two weeks before entry the discharge subsided, but the swelling remained Epigastric distress, gaseous eructations, crampy pain, fulness and regurgitation of food were prominent during the few weeks before entry

A physician made the diagnosis of abdominal neoplasm with metastasis to the umbilicus and sent the patient to the hospital

The familial social and past histories, as well as the system review, were non-contributory

The examination on this, the first, admission showed a small elderly woman appearing chronically ill Her temperature was 98.6 F, pulse rate, 80, respiration, 20, blood pressure, 200 mm of mercury systolic and 80 mm diastolic The skin was dry, warm and smooth, with loss of subcutaneous tissue There was limitation of motion of spine and hips The fundi showed arteriosclerotic and hypertensive changes The heart was enlarged and showed grade I apical and basal systolic murmurs The abdomen was rounded and distended, with hyperactive peristalsis In the midabdominal region there was minimal tenderness Palpation further revealed a poorly defined, firm, relatively fixed mass which was presumably attached to the firm, rounded, granulating, 10 by 20 cm lesion at the umbilicus Pelvic and rectal examinations disclosed no abnormality

The hemoglobin content was 80 per cent, the white blood cells numbered 7,000 per cubic millimeter, with polymorphonuclear leukocytes 60 per cent, the nonprotein nitrogen of the blood was 29 mg per hundred cubic centimeters, the total protein, 5.8 Gm per hundred cubic centimeters, the Hinton test was negative, two

3 Trimmingham, H. L., and McDonald, J. R. *Surg., Gynec. & Obst.* 80: 152, 1945

4 Rappoport, A. E., and Nixon, C. E. *Arch. Path.* 41: 388, 1946

stool specimens submitted to the guaiac test gave a negative result, a guaiac test of vomitus gave a 2 plus reaction, urine specimens concentrated to 1010 and showed only an occasional white blood cell. A roentgenographic series made to reveal metastatic growths showed only arthritic changes. A gastrointestinal series was made with use of a barium sulfate enema but reported as unsatisfactory. A biopsy specimen of the umbilical lesion taken at this time showed adenocarcinoma.

The course in the hospital with symptomatic and supportive therapy was relatively uneventful. It was felt that adenocarcinoma appearing at the umbilicus meant that wherever the primary lesion was it was inoperable, and therefore the patient was discharged to a nursing home on the thirtieth hospital day. The surgeons, in consultation, felt that operation was inadvisable.

Approximately six months later, October 23, the patient reentered the Boston City Hospital because of inability to eat solid food. Abdominal pain had become severe, and she vomited frequently, shortly after meals. To alleviate pain, morphine sulfate was being given twice daily at the time of this admission.

Examination at this time showed only more emaciation and slight disorientation. The temperature was 98.0 F, the pulse rate, 110, the respiratory rate, 12, the blood pressure, 135 systolic and 55 diastolic. The white blood cell count was 6,400, with 85 per cent polymorphonuclear leukocytes, 12 per cent lymphocytes and 3 per cent monocytes. The abdomen was boardlike. The umbilical lesion was fungating and had increased to 20 by 20 cm in size. It bled easily when rubbed with a throat stick.

Since the lesion was still felt to be secondary, the patient was again given symptomatic and supportive therapy. She gradually lapsed into a stuporous condition and quietly died on the third day after her second admission, approximately a year and a half after the initial development of symptoms.

The clinical diagnoses were adenocarcinoma of the umbilicus, primary site undetermined, ? terminal peritonitis, general arteriosclerosis, hypertrophic arthritis, arteriosclerotic heart disease with cardiac enlargement, normal sinus rhythm, compensated, class 2, complete absence of teeth.

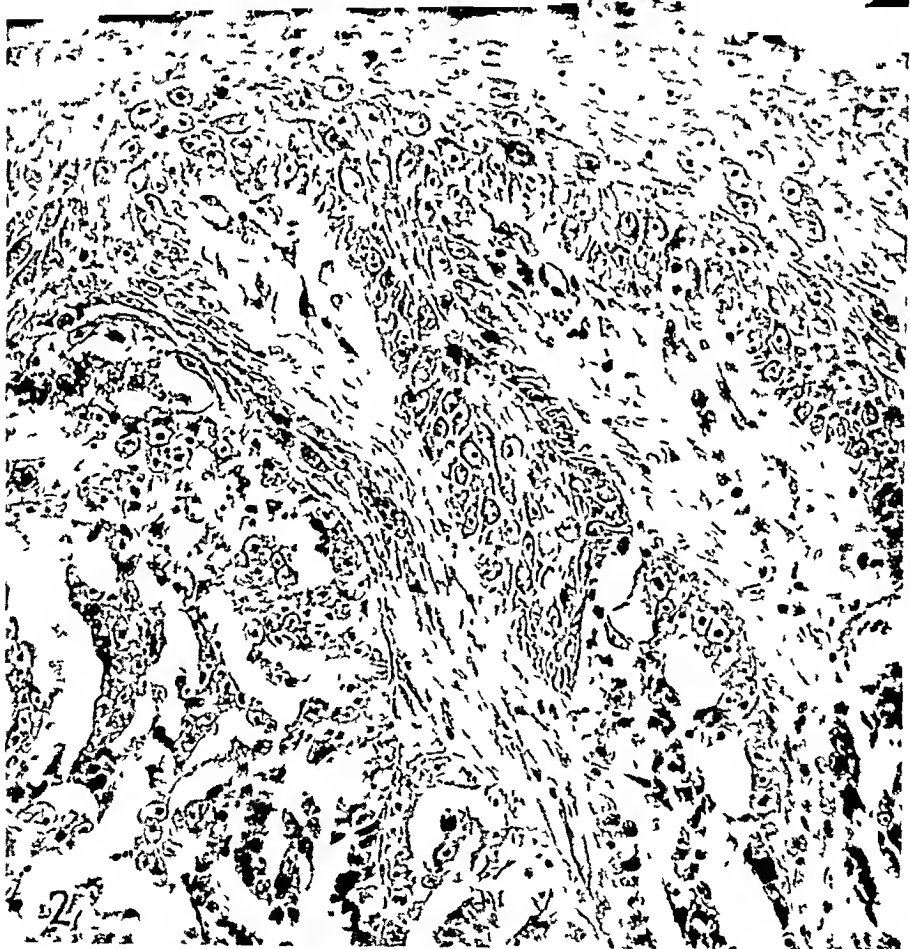
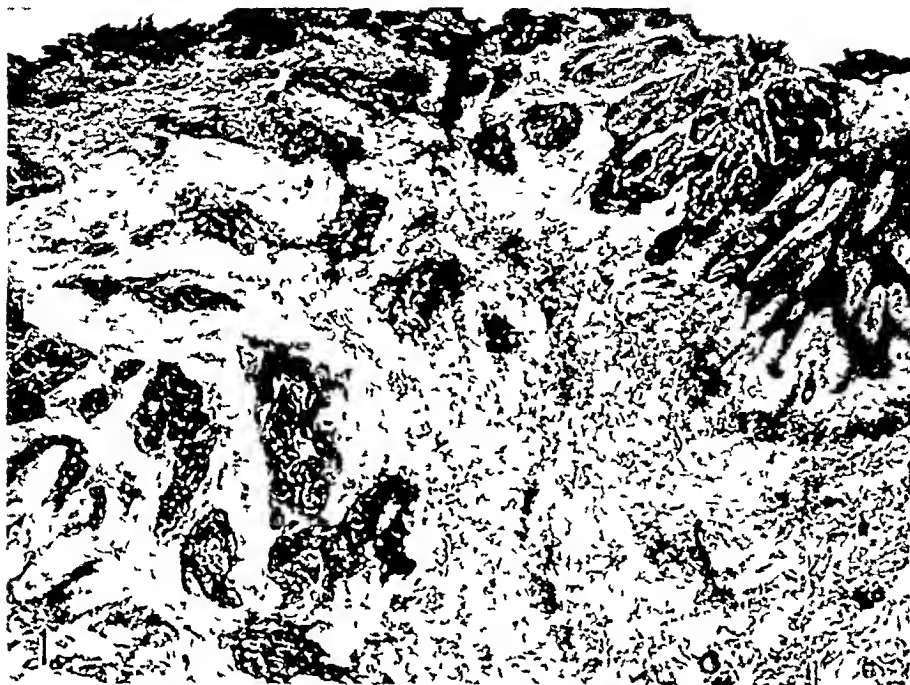
Autopsy—Only the pertinent gross and microscopic postmortem observations will be presented. The body was that of a fairly well developed, cachectic female weighing 90 pounds (41 Kg). In the umbilical area there was a blackened, crusted 20 cm area which exuded sanguinous material on pressure. No fistulous tract was demonstrable. There were no other external masses.

The peritoneal cavity was filled with thick, viscid yellowish green material collected into pockets, which were successively broken with manipulation. All intestinal loops were interadherent and also plastered against the abdominal walls by intervention of a gray-green and dark red fibrinopurulent material. The domes of the diaphragm were rendered adherent to the liver on the right and to the spleen on the left by the same material.

The gastrointestinal tract revealed only a 2.5 cm ulcer with a smooth base and rolled edges in the first portion of the duodenum.

No other visceral masses or tumors were found.

Microscopic Examinations—The biopsy specimen taken approximately six months before death showed a slowly growing, well differentiated adenocarcinoma made up of columnar and cuboidal cells, invading the subcutaneous tissue and associated with a marked overlying chronic inflammatory reaction. The tumor was forming glands and tubular spaces, which were separated from each other by fine strands of connective tissue. The tumor cells were large, with slight variation



(See legends on opposite page)

in size. The cell outlines were not clearly discernible. The cytoplasm was palely basophilic. A large vesicular nucleus occupied almost the entire cell volume. The nucleolus was dark and dense. Mitoses were rare. No remnants of omphalomesenteric duct or of urachus were discernible.

The section of tumor taken at autopsy six months later was similar to the biopsy specimen, but now there was evidence of more rapid growth. The gland spaces were not so well formed, there was more irregularity of cell size, with occasional multinucleate giant cells and more frequent mitoses. The associated inflammatory reaction was more intense.

Examination of the edge of the duodenal ulcer revealed only acute and chronic inflammatory reaction with no evidence of cancer.

In the subdiaphragmatic collagen were noted several lymphatic spaces which were either lined with or contained large pale vesicular cells with very large nuclei and dense nucleoli, similar to those seen in the original biopsy specimen. Mitoses were rare here also.

Final Anatomic Diagnoses—Adenocarcinoma of the umbilicus, metastasizing to subdiaphragmatic lymphatics, fibrinopurulent peritonitis, with old and fresh subdiaphragmatic abscesses and healed and acute perisplenitis, chronic duodenal ulcer, brown atrophy of the myocardium, benign nephrosclerosis, cystic left ovary.

Bacteriologic Examination—Culture of the peritoneal fluid showed *Bacillus coli*, *Streptococcus* with alpha hemolysis and *Clostridium welchii*.

COMMENT

Normally, as has been mentioned, the umbilicus is such a fundamentally simple structure that carcinoma has practically no starting place except in the squamous epithelial coat. However, remnants of the omphalomesenteric duct, whose most common anomaly every one is familiar with as Meckel's diverticulum, may be found occasionally as rests in the umbilical structures, as well as urachal rests. It is undoubtedly from these vestigial remnants that primary adenocarcinoma of the umbilicus originates. Although no vestigial remnants were found in the specimens obtained at biopsy and autopsy, it is possible that they were destroyed by the tumor and the associated inflammatory reaction.

In the case presented, death was due to extensive fibrinopurulent peritonitis, which undoubtedly resulted from previous fistulization of necrotic tumor, allowing free communication between the peritoneal cavity and the outside.

The only site of tumor extension was in the subdiaphragmatic lymphatics, where the tumor resembled the slowly growing adenocarcinoma of the original biopsy. The presence of tumor cells here,

EXPLANATION OF FIGURES

Fig 1—Low power detail of a section of an autopsy specimen, showing a margin of skin ulcerated by tumor and diffuse subcutaneous infiltration with nests of poorly differentiated adenocarcinoma. The associated inflammatory reaction is marked. $\times 60$

Fig 2—Higher power detail of a section of the biopsy specimen, showing the slowly growing, well differentiated adenocarcinoma invading the connective tissue of the umbilicus up to the germinal layer of the epithelium. $\times 360$

according to Wilcox and Greenblatt,⁵ is one of the expected findings in a cancer of this type which has progressed to dissemination. Other possible sites of metastases are the axillary, the inguinal and even the hilar lymph nodes, which are reached via the transdiaphragmatic lymphatics.

SUMMARY

There are embryologic and histologic bases for the development of primary adenocarcinoma of the umbilicus.

In the case of primary adenocarcinoma of the umbilicus presented, death was due to peritonitis, which probably resulted from fistulization of the necrotic tumor, allowing communication between the peritoneal cavity and the outside.

Early recognition of primary adenocarcinoma of the umbilicus is important because when correctly diagnosed the lesion represents a readily curable disease.

⁵ Wilcox, E. A., and Greenblatt, R. B. *Am J Surg* **34** 116, 1936.

LIPOMA OF THE MAMMARY GLAND

BELA HALPERT, M D, and MILLINGTON O YOUNG, M D, OKLAHOMA CITY

ADIPOSE tissue is a normal component of the mammary gland, yet a neoplasm consisting of adipose tissue alone, a lipoma, occurring in the mammary gland proper is exceedingly rare¹ This impression is amply confirmed by the available reports Menville,² who collected the literature on lipoma of the mammary gland, listed 24 cases from the Johns Hopkins Hospital, to which Geschickter³ added 6 According to Geschickter, "practically all of the lipomas were superficial, that is, anterior to the mammary gland proper" Adair, Pack and Farrior⁴ encountered 15 patients with lipoma of the mammary gland at the Memorial Hospital for the Treatment of Cancer and Allied Diseases and stated that the tumor was "usually retromammary" Among the patients of the Skin and Cancer Unit of the New York Post-Graduate Medical School and Hospital, de Cholnoky⁵ encountered 27 with lipoma of the mammary gland He did not state, however, the exact site of the growth The available information thus fails to reveal the number of cases in which lipoma actually arose within the substance of the mammary gland This paper records a case of lipoma of the mammary gland

REPORT OF A CASE

A 34 year old white woman was admitted to the University Hospitals, Oklahoma City, Jan 19, 1943, complaining of a mass which had been present in the left mammary gland for eight years It had gradually increased in size She had had some pain in the left mammary gland, associated with menses For four months prior to admission she had suffered from a dull aching in the left side of the chest and left shoulder, relieved by wearing a brassiere She was the mother of five children, four of whom were born after the mass was first noticed

At the time of admission the patient was obese and appeared to be in good health The only positive finding was a mass, about 10 by 8 cm, within the lower outer quadrant of the left mammary gland The mass was circumscribed, freely movable, fairly firm and lobulated It was not fixed to the skin or deeper structures There was no retraction of the nipple, dimpling of the skin, tenderness, redness or increased local heat The axillary lymph nodes were not palpable

From the Department of Pathology, University of Oklahoma School of Medicine

1 Spalding, J E Guy's Hosp Rep **94** 80, 1945

2 Menville, J G Am J Cancer **24** 797, 1935

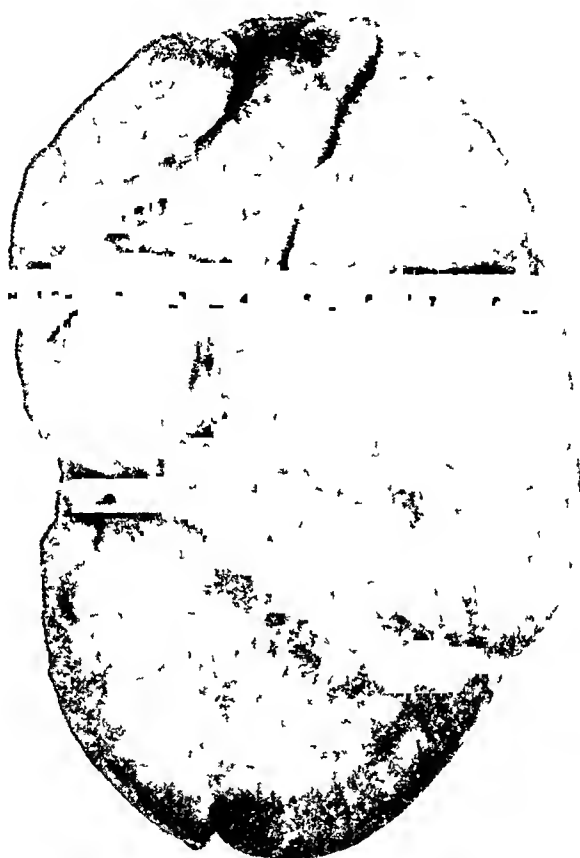
3 Geschickter, C F Diseases of the Breast, ed 2, Philadelphia, J B Lippincott Company, 1945, p 356

4 Adair, F E, Pack, G T, and Farrior, J H Am J Cancer **16** 1104, 1932

5 de Cholnoky, T Arch Surg **38** 79, 1939

The mass was thought to be a fibroadenoma. On January 22, a large lobulated and encapsulated "fatty tumor" replacing the lower half of the left mammary gland was excised (by Dr John W Cavanaugh). The postoperative course was uneventful, and the patient was discharged on January 28. Three and one-half years later there had been no recurrence of the growth.

The specimen consisted of a lobulated, encapsulated mass of adipose tissue, 14 by 9 by 8 cm, weighing 360 Gm (figure). The cut surfaces were composed of lobules of adipose tissue with delicate septums. Microscopic preparations stained with hematoxylin and eosin, representing many parts of the growth, disclosed



Lipoma of the mammary gland removed from a 34 year old white woman. The growth is composed entirely of various-sized lobules of adipose tissue.

various-sized lobules of adipose tissue cells with scant delicate septums containing blood vessels. Nowhere were any acini or ducts of the mammary gland seen. A delicate connective tissue capsule delimited the growth.

COMMENT

A focal or a diffuse increase of the adipose tissue surrounding the acini and ducts of one or both mammary glands is properly designated as lipomatosis. Such adipose tissue infiltration of the mammary gland is analogous to similar infiltration of the pancreas or the myocardium.

A growth immediately beneath the skin, near or overlying the mammary gland, should be classed as subcutaneous lipoma. True lipoma of the mammary gland is a focal aggregation of neoplastic adipose tissue cells delimited by a capsule and located within the mammary gland proper.

SUMMARY

A large lipoma of the left mammary gland was observed in a 34 year old white woman. A survey of the literature disclosed that true intramammary lipoma is exceedingly rare.

ACUTE BRONCHOPNEUMONIA DUE TO ASPERGILLUS FUMIGATUS FRESENIUS

Report of a Case, with a Description of Acute and Granulomatous Lesions Produced by the Fungus in Rabbits

NORMAN S. COOPER, M.D., NEW YORK

THE LITERATURE contains fairly numerous references to pulmonary aspergillosis. In the great majority of the reported cases the causative organism was *Aspergillus fumigatus*. In those instances in which the diagnosis was made during life the disease usually manifested itself in a syndrome clinically and roentgenologically indistinguishable from that of chronic pulmonary tuberculosis. Aspergillosis was identified when the causative organism was isolated from the sputum in the absence of the tubercle bacillus. Anatomically, in the reported cases, chronic pulmonary aspergillosis was characterized by the formation of granulomas (often with giant cells), by fibrosis and by cavitation.¹

In the older literature a few cases of a more acute type of pulmonary aspergillosis were reported.² These cases were usually diagnosed only at autopsy. The lesions consisted of colonies of the mycelia and spores of *Aspergillus* surrounded by an area of necrosis, with or without a delimiting zone of polymorphonuclear neutrophils. A search of the recent literature has failed to reveal any anatomic descriptions of acute pulmonary aspergillosis.

Acute aspergillar bronchopneumonia was an unexpected finding in a recent autopsy at this hospital.

REPORT OF A CASE

A 45 year old, white, Italian-born barber was admitted to the New York Hospital complaining of severe epigastric pain. A chronic duodenal ulcer had been diagnosed by fluoroscopic and roentgen examinations on previous admissions. Laparotomy was performed on the day of admission, and a ruptured duodenal ulcer was plicated.

The patient's temperature rose to 40.3 C (104.5 F) on the third postoperative day, and thereafter it ranged between 37.7 and 40.4 C (99.8 and 104.7 F) despite the administration of sulfadiazine (a blood level of 68 mg per hundred cubic centimeters was attained). On the sixth hospital day laparotomy was done a second time, pus was seen oozing from the site of the previous plication. On the fourteenth hospital day jejunostomy was performed to permit feeding by tube.

From the New York Hospital and the Department of Pathology, Cornell University Medical College.

1 (a) Renon, L. *Étude sur l'aspergillose chez les animaux et chez l'homme*, Paris, Masson & Cie, 1897. (b) Watjen, J., in Henke, F., and Lubarsch, O. *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1931, vol. 3, pt. 3, p. 481.

2 Saver, F. *Pneumomykosis Aspergillina*, Jena, Gustav Fischer, 1900. Watjen.^{1b}

Subsequently the patient became weaker and more lethargic. The original operative wound, which had never healed, drained bile-containing fluid.

Since the seventh hospital day the patient's respirations had ranged between 32 and 40 per minute, and there was occasional cyanosis. On the fifteenth hospital day a nonproductive cough, rhonchi and rales developed and persisted. The patient became cyanotic and stuporous on his twentieth hospital day and died on the twenty-first day.

Roentgenograms of the chest made on the second, fourth and sixth hospital days were interpreted as showing atelectasis and pneumonic consolidation at the base of the right lung.

Autopsy—There was generalized peritonitis. Immediately beyond the pylorus a gaping circular perforation, 12 mm in diameter, was seen in the anterior wall of the duodenum. There were silk sutures in the indurated margins of the perforation.

There were fibrinous adhesions between the right lung and the diaphragmatic pleura. The lungs together weighed 1,530 Gm. On cut surfaces the upper and lower lobes of both lungs were studded with firm, white, raised nodules, 1 to 2 mm in diameter. From many of the nodules pus exuded on pressure. The process was most marked in the upper lobe of the right lung. The middle lobe of this lung was atelectatic, though practically free from the nodular lesions.

Microscopic Observations—The lungs contained many small abscesses in bronchi and immediately adjacent alveoli (fig 1). Alveoli around the abscesses contained neutrophils, mononuclear phagocytes and small amounts of fibrin. In the centers of the abscesses were what appeared to be sporulating hyphae. These were faintly basophilic, translucent and about 7 to 10 microns in diameter and 30 to 120 microns long. Many of them had joints resembling those of a bamboo pole, and some branched one or more times (fig 3). A few basophilic round bodies were free in the abscesses. The hyphae had the same appearance in hematoxylin-eosin, Brown,^{2a} Mac-Callum^{2b} and Giemsa stains. No bacteria were seen.

Culture of the peritoneal exudate yielded hemolytic *Staphylococcus aureus*. Cultures of material from the upper lobe of the right lung, made originally in liver-dextrose broth and transferred later to Sabouraud's agar and blood agar, showed *Aspergillus fumigatus* Fresenius in pure growth except for one colony of *Staphylococcus albus*. Dr. Kenneth B. Raper, of the Agricultural Research Administration, United States Department of Agriculture, identified the fungus.

EXPERIMENTS

Inoculations showed that this strain of *A. fumigatus* is pathogenic for rabbits. The fungus was transplanted to dextrose agar plates, and the subcultures were grown at 30 C for five to seven days. A heavy suspension of spores of the organism was prepared by washing off the surfaces of agar plates with a small quantity of Locke's solution. The rabbits were anesthetized with ether, and 1 cc of the spore suspension was injected intratracheally. The animals were maintained under anesthesia with their heads elevated for an hour after the operation. All of them appeared to be normal postoperatively. They were killed at varying periods.

Twenty-four and forty-eight hours after the intratracheal injections sections of the lungs showed a histologic picture (fig 2) identical with that encountered

2a Brown, J. H., and Breun, L. *Bull. Johns Hopkins Hosp.* 48:69, 1931.

2b Mallory, F. B. *Pathological Technique*, Philadelphia, W. B. Saunders Company, 1938, p. 274.

in the autopsy described in the foregoing pages except that only a few colonies of the fungus were seen. In these the hyphae were paler staining, showed a lesser tendency to branch and were smaller than those in the reported case.

In animals killed six to twenty-six days after inoculation the acute broncho-pneumonic character of the lesions had disappeared. There was a granulomatous

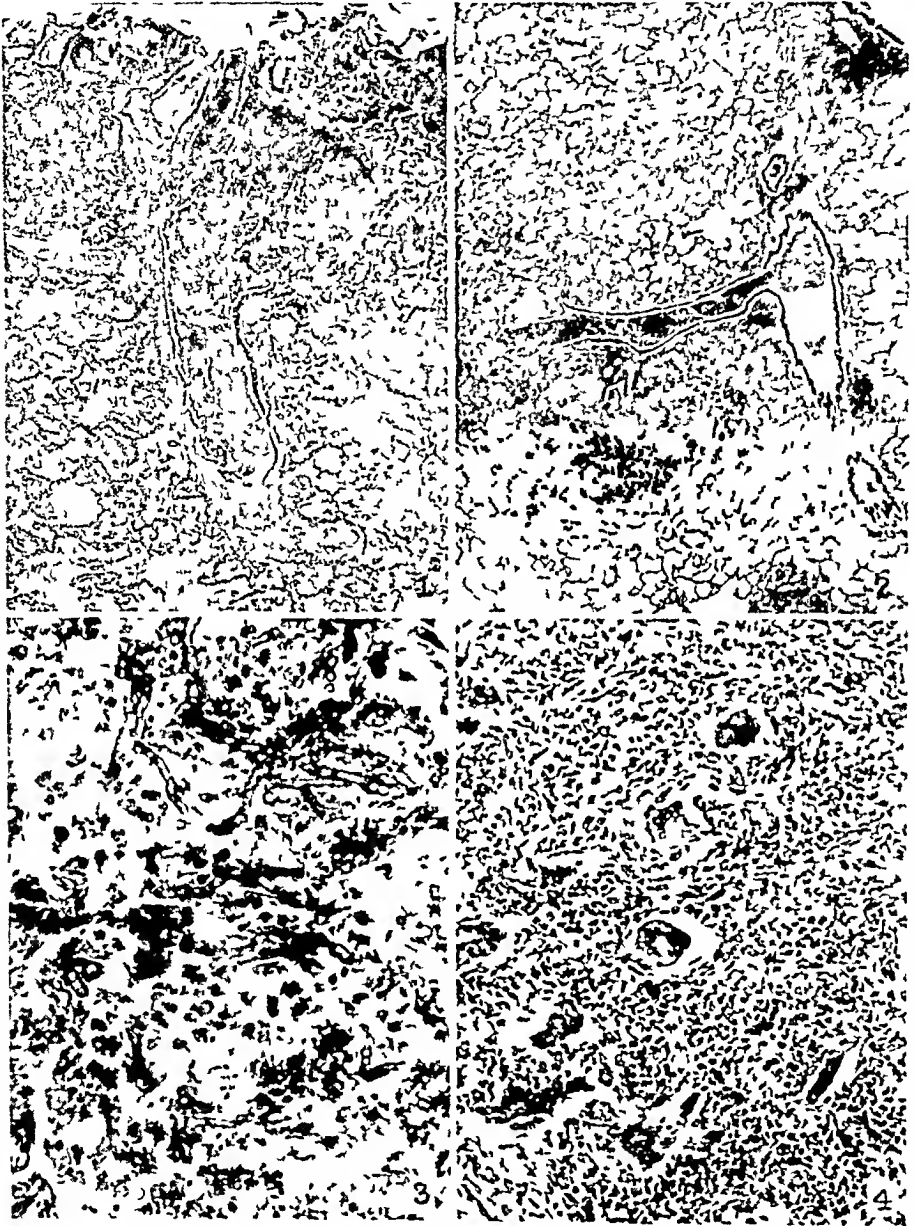


Fig 1—Low power view of a section from the upper lobe of the right lung of the patient

Fig 2—Low power view of a section of the lung of a rabbit twenty-four hours after intratracheal inoculation of *A. fumigatus*

Fig 3—High power view of a section from the lung of the patient, showing the hyphae of *A. fumigatus*

Fig 4—High power view of the granulomatous reaction in the lung of a rabbit six days after intratracheal inoculation of *A. fumigatus*

reaction, with masses of epithelioid cells, lymphocytes and giant cells (fig 4). There were large necrotic areas, bordered by collagen. The lesions were slightest in the animal which had been allowed to survive longest. No lesions at all were found in the lungs of a rabbit which was killed seventy-three days after inoculation. No organisms were seen in the sections from this group of animals, but *A. fumigatus* in pure growth was obtained in cultures of material from the lungs in every instance except that of the rabbit which had been allowed to survive for seventy-three days.

Rabbits which were given 5 cc of the spore suspension intravenously died within twenty-four hours. Multiple abscesses were found in the heart, the lungs and the liver. Postmortem blood cultures showed *A. fumigatus* in pure growth, but no hyphae were seen in histologic sections.

COMMENT

For some years, a controversy existed over whether pulmonary aspergillosis was ever a primary disease. Because of the ubiquity of *Aspergilli* and the rarity of aspergillosis, many authors found it difficult to accept the thesis that the fungi could invade undamaged lung tissue. Others pointed out that repeated exposure to large numbers of spores might be necessary for infection. Renon¹ observed aspergillosis in wigmakers and pigeon feeders, an interesting fact being that the latter took grain into their mouths, chewed it and then forced it into the mouths of pigeons. The wigmakers used corn meal for freeing the hair with which they worked from oil. Corn meal, grain and the mouths of pigeons are all rich sources of *Aspergilli*.

In many of the cases reported in the older literature there were other coexisting lesions of the lungs, such as tuberculosis, infarcts, bacterial pneumonia or pulmonary abscess. In most of those in which the aspergillosis was called primary, attempts to rule out other lesions, particularly tuberculosis, by cultural methods were inadequate. In at least one of Renon's patients whose aspergillosis was reported as primary, it was subsequently shown to be associated with tuberculosis.³ More recently, few cases of pulmonary aspergillosis in which autopsies were made have been reported. In 1923 Lang and Grubauer published the details of a case in which aspergillosis appeared to be secondary to bronchiectasis.⁴ In 1926 Macaigne and Nicaud⁵ described a case of chronic aspergillosis in which careful search failed to reveal tuberculosis or other complicating lesions. Most authors have accepted this work as demonstrating that pulmonary aspergillosis can occur as a primary disease. However, the possibility remains that the traces of a preexisting disease might have been obliterated by the unusually extensive fibrosis which was present.

In the case reported here, there was no evidence of the presence of any pulmonary lesion other than aspergillosis, but the patient had had peritonitis for at least the last two weeks of life. Watjen^{2b} discussed the possibility that debility may be the predisposing factor in otherwise primary pulmonary aspergillosis.

³ Sergeant, cited by Macaigne and Nicaud.⁵

⁴ Lang, F. J., and Grubauer, F. *Virchows Arch f path Anat* **245** 480, 1923.

⁵ Macaigne, M., and Nicaud, P. *Presse méd* **34** 401, 1926.

In the experiments described in this report the seeding of the organisms in the lungs of the rabbits may have been facilitated by prolonged ether anesthesia. Under these conditions the organism isolated from the patient's lungs proved capable of inducing acute bronchopneumonia in healthy rabbits. However, the disease soon became granulomatous, and it later disappeared entirely. At no time did the animals which had received intratracheal injections of the suspension of spores appear to be ill.

SUMMARY

A case is presented in which acute bronchopneumonia due to *A. fumigatus* was an unexpected finding in a debilitated patient. No lesions other than those due to aspergillosis were found in the lungs. The organism was seen in histologic sections and isolated in virtually pure culture from the lungs. Similar bronchopneumonia was produced in rabbits by intratracheal injections of a suspension of spores of the organism thus obtained, and the fungus was obtained in pure culture again from the rabbits' lungs. After the lapse of several days the lesions in the rabbit became granulomatous, eventually they disappeared.

Laboratory Methods and Technical Notes

SILVER IMPREGNATION OF SPIROCHETES IN TISSUE SECTIONS

Description of a New Technic

MADUREIRA • PARÁ, M D, RIO DE JANEIRO, BRAZIL

NUMEROUS modifications of the original Levaditi¹ (1905) method of demonstrating spirochetes in tissues have been proposed, all aimed at simplifying the procedure, making it more rapid or offering a wider range of application

A modification suggested by Warthin and Starry² for staining paraffin-mounted tissue sections deserves special mention. The sections, fixed on cover slips, are sensitized with ferrous ammonium sulfate or with ferric alum (iron and potassium sulfate) before being treated with silver nitrate. Impregnation is brought about through selective capillary action, since the cover slip, with the section mounted on it, is covered by a second cover slip of equal size before it is placed in the silver solution. Another interesting technic, applicable either to frozen or paraffin sections, has been devised by Dieterle³. It consists of coating the section with gum mastic, thus giving it the physical characteristics of a true tissue block. However, the practical difficulties and the failures which often result when one is employing these two technics render them unsuitable for ordinary diagnostic purposes.

Krajian⁴ described an improvement on Dieterle's method, but one applicable only to frozen sections. Steiner and Steiner⁵ suggested modifications which are applicable only to paraffin sections mounted on slides. In my experience the Steiner modification represents the easiest and most reliable method so far described. However, the necessity of using sodium and potassium tartarate as well as gum mastic limits its application, since neither of these substances is commonly available in histologic laboratories. Furthermore, gum mastic is, on occasion, difficult to obtain.

THE COLLOIDAL SILVER-LITHIUM IMPREGNATION TECHNIC

After making many attempts to find a technic for impregnation which would be reliable, easily executed and time saving, I finally developed a new method which can be applied in either of two processes, one slightly more complex and consequently more time consuming than the other. Both are recommended, since the results obtained by either proc-

From the Histopathological Section of the National Yellow Fever Service

1 Levaditi, C. *Compt Rend Soc de biol* **59** 326, 1905

2 Warthin, A. S., and Starry, A. C. *Am J Syph* **4** 97, 1920

3 Dieterle, R. R. *Arch Neurol & Psychiat* **18** 73, 1927

4 Krajian, A. A. *Histological Technic*, St. Louis, C. V. Mosby Company, 1940, p. 163

5 Steiner, G., and Steiner, G. *J Lab & Clin Med* **29** 868, 1944

ess are in complete accord with those obtained by the original Levaditi or the Steiner method

This new process is applied to paraffin-embedded sections mounted on slides. Preferably tissues should have been fixed in solution of formaldehyde U S P diluted 1:10 in saline solution

BASIC METHOD

Solutions Required—1 Uranium nitrate. A 1 per cent solution in water. This is stable at room temperature.

2 Silver nitrate. A 15 per cent solution in water. This can be freshly prepared or stored in the dark at room temperature.

3 Silver nitrate-lithium carbonate solution. This is prepared as follows: Heat 100 cc of a 0.2 per cent silver nitrate solution almost to boiling and add 20 cc of a cold saturated (about 13 per cent) solution of lithium carbonate in water. The saturated lithium carbonate solution is preferably prepared a few days before use. Heat the mixture gradually and allow it to boil for one or two minutes. The resulting opalescent solution is passed through ordinary filter paper, and the clear filtrate obtained is stored in a refrigerator, where it will keep for a maximum period of one month.

4 Rosin (colophony). A 5 per cent solution in absolute alcohol. This solution should be preserved in a refrigerator.

5 Levaditi's reducing solution. This has the following formula:

| | |
|--------------------------------|----------|
| Picrogallie acid | 4.0 Gm |
| Solution of formaldehyde U S P | 5.0 cc |
| Distilled water | 100.0 cc |

Procedure—1 Cut paraffin-embedded sections of tissue 4 to 6 microns thick and fix on slides. Remove the paraffin and hydrate in the usual manner.

2 Place the sections in the uranium nitrate solution and leave at room temperature for thirty minutes.

3 Wash rapidly in distilled water.

4 Treat with the silver nitrate solution for two hours in an incubator at 56 C, replacing the solution at the end of the first hour with fresh silver nitrate. Or, as an alternative, the sections may be treated at 37 C overnight without changing the solution.

5 Wash rapidly in distilled water.

6 Transfer to the silver-lithium colloidal preparation and treat for one hour at room temperature. The mixture is prepared in a 50 cc Coplin jar immediately before using, in the following manner:

| | |
|---|-------|
| Silver nitrate-lithium carbonate solution | 45 cc |
| 5 per cent alcoholic solution of rosin | 5 cc |

The rosin solution is added rapidly to the silver nitrate-lithium carbonate solution. The mixture is shaken gently, and a milky colloidal suspension results.

7 Transfer the sections directly to the Levaditi reducing solution and allow them to stand at room temperature for ten to fifteen minutes.

8 Wash rapidly in distilled water.

9 Examine the slide under the microscope to determine whether the tissue has been properly impregnated. If not, treat the section rapidly with absolute alcohol.

and repeat step 6 for ten minutes, then follow with another reduction lasting five to ten minutes

10 Dehydrate and mount in Canada balsam

Sections impregnated in this manner have the yellow-brown color characteristic of sections stained by Levaditi's original method. The cell nuclei stain brown, and the spirochetes stain black.

It occasionally happens that some sections fail to become well impregnated, this is shown by an excessive prominence of connective tissue or by the formation of a fine precipitate. Generally it is due to a faulty fixing solution. In such cases new sections are cut from the paraffin block, fixed on slides, the paraffin removed and the section hydrated in the usual manner as far as 50 per cent alcohol. The slides are then placed in a 2 per cent solution of rosin in absolute alcohol for one hour at room temperature. They are then washed in absolute alcohol, rehydrated and impregnated by the regular process.

VARIATIONS IN THE METHOD

In order that this method might be made as practical as possible, other reagents have been tested which may be used as substitutes for certain chemicals employed in the basic process. When used in the manner described, each functions in a satisfactory manner. Those which were found to give results almost as satisfactory as the basic reagents are listed here.

Substitutes for Uranium Nitrate as Mordant—1 Ferrous ammonium sulfate. A 4 per cent solution in water is used. The sections are treated for forty-five minutes at 56 C.

2 Sulfur water. This is prepared as follows. To 200 cc of distilled water add 10 cc of a 5 per cent solution of anhydrous sodium bisulfite and 10 cc of normal hydrochloric acid. The solution, which has the odor of sulfur dioxide, is kept in a tightly stoppered flask. Sections are treated with this solution for ten to fifteen minutes at room temperature.

3 Oxalic acid. A 1 per cent solution in water is used. Sections are treated for twenty minutes at 56 C.

4 Copper sulfate. A 0.5 per cent solution in water is used. Sections are treated for thirty minutes at room temperature.

5 Potassium permanganate. This is used in the form of a 1:5,000 dilution in water. Sections are treated for five minutes at room temperature.

Substitutes for Silver Nitrate—Lithium Carbonate Solution in the Sensitizer—1 Colloidal silver tartrate preparation. The basic method is carried out through step 5. The sections are then transferred to the following mixture. To 40 cc of Steiner's double tartarate-silver nitrate solution, contained in a Coplin jar, 10 cc of a 5 per cent solution of rosin in alcohol is added rapidly. The sections are treated with the resulting milky fluid for one hour at room temperature. The basic process is then continued, beginning with step 7.

2 Oxidation. This variation is of particular interest, since sensitization of the impregnated section is accomplished by the selective action of a normal organic secretion without the need of a silver salt. The basic method is carried out through step 4. The sections are then transferred to a sensitizing solution consisting of

40 cc of a 10 per cent dilution of fresh ox bile in water and 10 cc of a 5 per cent solution of rosin in alcohol. The sections are treated for one hour at room temperature in the resultant yellowish milky fluid, and then the routine process is continued, commencing at step 7.

Substitutes for Rosin in the Sensitizer—1 Gum mastic. The basic method is followed through step 5. The sections are then treated for one hour at room temperature with the following milky preparation. To 40 cc of the silver nitrate-lithium carbonate solution 10 cc of a 2.5 per cent solution of gum mastic in alcohol is added, rapidly. The flask is shaken gently. Steiner's silver tartrate solution can be used in place of the silver-lithium solution. After this treatment of the sections the routine process is resumed, beginning with step 7.

2 Ox bile. The basic method is followed through step 5. The sections are then sensitized for one hour at room temperature in a greenish yellow and opalescent mixture of 45 cc of silver nitrate-lithium carbonate solution and 5 cc of fresh ox bile. The bile is added to the silver-lithium solution and mixed by shaking. If desired, Steiner's silver tartrate preparation can be used in place of the silver-lithium solution. After this treatment the routine process is resumed from step 7.

3 Balsam of Tolu. The basic method is followed through step 5. The sections are then sensitized for one hour at room temperature in a milky mixture of 45 cc of silver nitrate-lithium carbonate solution and 5 cc of a 1 per cent solution of balsam of Tolu in alcohol. Following this treatment the routine process is resumed from step 7 on.

Substitute for Solution of Formaldehyde-Pyrogallie Acid Reducing Agent—Hydroquinone. A solution of 1 Gm of hydroquinone in 60 cc of distilled water can be substituted for the formaldehyde-pyrogallie acid reducing agent. The sections are treated for ten to fifteen minutes at room temperature.

RAPID METHOD

Solutions Required—1 Silver nitrate. A 1 per cent solution in water. Preferably this should be prepared just before using.

2 Lithium carbonate. A dilution is made consisting of 1 cc of a cold saturated aqueous solution (about 13 per cent) in 165 cc of distilled water. This diluted solution will keep for about five days at room temperature. If lithium carbonate is not available a 0.1 per cent solution of sodium and potassium tartrate may be used with equally satisfactory results.

3 Rosin. A 2 per cent solution of rosin in alcohol is used. This is made as follows. A 50 per cent solution of rosin in absolute alcohol is prepared. After the rosin is completely dissolved, it is passed three times through filter paper. It may be stored in the refrigerator for long periods. Just before use it is diluted twenty-five times with alcohol to a final concentration of 2 per cent.

4 Levaditi's reducing solution. This is the same as that used for the "basic method."

Procedure—1. Paraffin-embedded sections, cut 4 to 6 microns thick, are fixed on slides, the paraffin is removed, and the sections are hydrated in the usual manner as far as distilled water.

2 The sections are transferred to the following reagent, prepared just before use: silver nitrate solution, 30 cc, lithium carbonate solution, 10 cc, rosin solution, 10 cc. The lithium carbonate and silver nitrate solutions are first mixed in a Coplin jar. To this opalescent mixture the rosin solution is added rapidly, and the mixture

is well shaken. The sections are treated in this milky preparation for one hour at 56 C.

3 The sections are transferred directly to a reducing mixture with the following composition: Levaditi's reducing solution, 50 cc; rosin solution, 5 cc. The sections are treated in this preparation for seven to ten minutes at room temperature.

4 Wash in two or three baths of 90 per cent alcohol for five minutes each.

5 Wash rapidly in distilled water.

6 Replace in the silver-lithium-rosin solution used in step 2 for an additional fifteen minutes at 56 C.

7 Transfer directly to the reducing solution used in step 3 for a second treatment of three to five minutes at room temperature.

8 Wash in two 90 per cent alcohol baths, two minutes in each bath.

9 Dehydrate and mount in Canada balsam.

In case unsatisfactory results are obtained the corrective procedure described for the basic process may be applied.

COMBINED PROCESS FOR DEMONSTRATING SPIROCHETES AND PATHOLOGIC CHANGES

Frequently it is desirable to study the pathologic changes in the tissue preparations as well as to demonstrate the presence of spirochetes. For this purpose the "rapid process" can be used with two variations: (1) the omission of rosin in the reducing solution and (2) the subsequent use of a contrast stain. In detail the process is as follows:

1 The rapid process is carried out through step 8 with the exception that the reducing solution used in steps 3 and 7 contains no rosin.

9 Wash in distilled water.

10 Stain the sections for ten to fifteen minutes in Harris' hematoxylin.

11 Pass rapidly through the diluted lithium carbonate solution.

12 Wash in running water.

13 Stain one to three minutes in Altmann's acid fuchsin which has been diluted 1:5 in distilled water just before using.

14 Differentiate in absolute alcohol for five minutes.

15 Dehydrate and mount in Canada balsam.

By this process the cytoplasm is stained light red, the nuclei dark violet, the connective tissue bright red or ochre yellow and the spirochetes black.

Satisfactory results can also be obtained with other counterstains as follows:

Van Gieson's stain. This is used in place of diluted Altmann's fuchsin. The staining time is one minute.

Meyrick and Harrison stain. This stain was originally recommended by Meyrick and Harrison⁶ as a background stain for their application of Gram's method to paraffin-embedded tissues. It is composed of 15 parts of an aqueous 1 per cent solution of neutral red and 1 part of carbolfuchsin (a mixture of 9 cc of an alcoholic 10 per cent solution of basic fuchsin and 90 cc of 5 per cent phenol in water). The mixture remains unchanged for long periods. It is used as follows:

⁶ Meyrick, L. D., and Harrison, C. V. *J. Path. & Bact.* 54: 517, 1942.

- 1 The rapid process is followed through step 8 except that the reducing solution used in steps 3 and 7 has no rosin
- 9 Wash in distilled water
- 10 Stain for ten minutes
- 11 Differentiate rapidly in absolute alcohol
- 12 Dehydrate and mount in Canada balsam

By this process the cytoplasm is stained light red, the nuclei bright red, the connective tissue dark red or greenish yellow and the spirochetes black.

COMMENT

The accurate and rapid demonstration of spirochetes in tissue sections is of distinct histologic value. Not only can lesions associated with the presence of these organisms be studied, but, in addition, the demonstration of the organisms simplifies recognition of various visceral lesions whose polymorphous nature would not otherwise permit a definite pathologic diagnosis. The value of such a technic is enhanced when the histopathologic material available for examination is limited. Such is often the case with viscerotomy specimens.

The use of the method described is practical, since the necessary reagents are inexpensive, easily obtained and commonly used in histologic technic.

The new method of silver impregnation, based on the use of a colloidal suspension of lithium carbonate and silver nitrate, can be utilized in various ways, the principal modification being the "rapid method." The latter technic, which dispenses with a mordant and reduces the essential procedure to a double process of impregnation and reduction in a colloidal medium, can be performed in two hours. In addition, aniline counterstains can be used.

Other variations of the basic method are described for use when chemicals necessary for the standard method are not available. These alternatives are listed in the order of preference.

It is worth while to emphasize the possibility of using ox bile in the silver impregnation of spirochetes in tissue sections, since this has been found to be an excellent substitute either for the rosin or for the lithium-silver complex. Of all reagents used, bile is the only one in which this dual capacity has been demonstrated. Furthermore, it is easy to obtain.

I have observed comparable and regular results with both the basic and the rapid method, not only when these results were checked against those given by the original Levaditi method for tissue blocks in solution of formaldehyde, but also when they were checked against those given by the Steiner method. For this study I used viscerotomy, autopsy and experimental material, containing *Treponema pallidum*, *Treponema penitens* or *Leptospira icterohaemorrhagiae*.

From a practical point of view either of the two methods described can be used. However, if there is no great urgency in arriving at a diagnosis, or if there is only a small amount of material available it is wise to use the basic method, carried out at 37 C., which gives certain and uniform results with any type of tissue.

SUMMARY

A new method for silver impregnation of spirochetes in paraffin tissue sections mounted on slides provides accurate and rapid demonstration of these organisms. There are two variations of the method, the "basic method," whose fundamental reagents are uranium nitrate, silver nitrate, lithium carbonate, rosin and Levaditi's reducing solution, and a "rapid method," in which a double process of impregnation and reduction avoids the use of a mordant. The fundamental principle involved in both technics is the use of a lithium-silver complex in a colloidal medium to obtain selective impregnation of the spirochetes.

Possible variations in the technic give a wider range of application and practicability.

Books Received

HUMAN EMBRYOLOGY By Bradley M. Patten, professor of anatomy in the University of Michigan Medical School. Pp 776, with 1,366 drawings and photographs grouped as 466 illustrations, 53 in color. Price \$7. Philadelphia and Toronto: The Blakiston Company, 1946.

The book is planned in such a way that each subject is handled in a stimulating manner to emphasize its importance in the practice of medicine. Developmental processes are presented as a succession of dynamic phases in a continuum, not as widely separated steps. Primordial stages in the morphogenesis of the embryo, the mechanism of vesicular implantation in the uterus, and the concomitant changes in the reproductive organs of the mother, which are so important in gynecology and obstetrics, have received particularly careful attention. Among the other features emphasized are the following: the advanced stages in organogenesis, basic to an understanding of the plan of the body as demonstrated in gross anatomy, the histogenesis of some of the major organs, calculated to explain the architecture of viscera as encountered in microscopic anatomy, the more common developmental anomalies, described because of their clinical importance. A special chapter is included to facilitate correlation of text and laboratory study. The contents of the volume record its broad sweep: reproductive organs, gametogenesis, the sexual cycle and fertilization, cleavage, formation of germ layers and establishment of the embryonic body, early differentiation of the body and establishing of organ systems, fetal membranes and placenta, age, growth and changes in external form of the body, twinning, double monsters and teratology, integumentary system, connective tissues and skeletal system, muscular system, development of the nervous system, organs of special sense, development of the nose, jaws and teeth, development of the digestive and respiratory systems, body cavities and mesenteries, ductless glands and pharyngeal derivatives, development of the urogenital system, development of the circulatory system. Forty-two pages are devoted to a bibliography which selectively lists the major sources of the information discussed. There are 1,366 drawings and photographs, grouped as 466 illustrations. Many of the illustrations are original drawings by the author, of the total number, 53 are in color. The textbook contains also serviceable graphs and semidiagrammatic figures illustrating skeletal, visceral and general body growth, the bases of anomalous development of organs and of persistence of vestigial structures. The text is readable, it flows with a facility which has pleased all readers of Professor Patten's scholarly manuals and journal articles. While "Human Embryology" is a new book, it carries every sign of maturity, it is the outgrowth of rich experience in teaching and in research achievement.

DR F. G. GADES, PATHOLOGISK-ANATOMISKE LABORATORIUM I BERGEN. Meddelelser 1940-1941 and 1942-1945. Various pagination. 1942 and 1946.

NOUVELLES ETUDES CLINIQUES ET BIOLOGIQUES SUR LA PATHOLOGIE DU FOIE By Étienne Chabrol, professeur de Clinique Médicale à la Faculté de Paris. Membre de l'Académie de Médecine. Paper. Pp 182, with 24 figures. Paris: Masson et Cie, 120 Boulevard Saint-Germain, 1946.

This monograph is composed of twenty-one lectures on selected topics in disease of the liver. The first three chapters constitute an interesting historical introduction to the contributions of the French in the early descriptions of hepatic disease. The remaining discussions center about case presentations. Two lectures touch on epidemic hepatitis, but the author appears unaware of the important recent

contributions from other countries. The illustrations give little information. The book is inaccurately titled in view of the fact that it is chiefly a review of previous publications of the author and his colleagues.

QUANTITATIVE CLINICAL CHEMISTRY. INTERPRETATIONS. By John P. Peters, M.D., M.A., professor of internal medicine, Yale University School of Medicine, and Donald D. Van Slyke, Ph.D., Sc.D., member of the Rockefeller Institute for Medical Research. Volume I. Second edition. Pp. 1041, with 62 illustrations. Price \$7. Baltimore: Williams & Wilkins Company, 1946.

The long-awaited second edition of this monumental handbook of clinical chemistry has finally arrived. At least part of it has, for the material covered is so vast that only the sections on total metabolism, carbohydrate, lipids and proteins could be included in this first volume of interpretations. The remaining subjects will be included in a later second volume, whereas methods will constitute a third. As it is, this small portion comprises 1,041 pages, compared with 1,264 of the complete first edition. It is principally written by Peters.

The subject of carbohydrates, which in the first edition was covered in probably the least authoritative chapter, is in this edition brilliantly expounded. The discussion of the biochemistry of intermediate carbohydrate metabolism, the role of insulin and other hormones and the vicissitudes of the blood glucose concentration and the clinical story of diabetes demonstrate a mature, broad-minded blending of the sciences of biochemistry, physiology, endocrinology, nutrition and clinical medicine. The assisting hand of the trained biochemist and carbohydrate authority C. N. H. Long is visible throughout the whole section.

The chapter on lipids has a similar wide scope, but the still uncrystallized state of the interpretation of the clinical variation of the individual blood lipids is disappointing, for one always imagines that authors of a monograph can bring order out of the chaos of the many controversial and mutually contradictory physiologic and clinical studies. Also, one hopes in vain that the survey of the 978 papers on which the exposition of lipids is based can lead to a clear picture of the metabolism of lipids in hepatic and other diseases. But the fault, of course, is not with the authors.

The steroid hormones are discussed all too briefly in 12 pages.

Part IV covers the subject of the metabolism of nitrogen in 6 chapters. Along with the retained description of plasma proteins and urea and the clinical story of Bright's disease—which were among the outstanding features of the first edition—is offered an up-to-date and authoritative elucidation of the role of protein nutrition in disease processes. Here the broad experience of both authors is easily recognizable. The chapter on purines has relatively little new material except for the isotopic studies of Schoenheimer.

There is little doubt that the book will be received with as much acclaim as the first edition, and deservedly so. Yet in spite of the broad biochemical and clinical experience of the authors and their indefatigable compilation, the limitations of a book with such ever-widening scope written by single authors is evident from the fact that there are few references after 1943.

HEPATITES RARES. By Maurice Loeper, professeur de Clinique Medicale a la Faculte de Medecine de Paris, et membre de l'Academie de Medecine. Paper. Pp. 214, with 17 figures. Price 290 francs. Paris: Masson et Cie, 1946.

In this monograph rare diseases of the liver are discussed in 21 chapters, together with some complications of hepatic disease and certain aspects of hepatic function. The illustrations are poor, and the bibliographies are brief. The book is well written, interesting, informative and at times provocative.

News and Notes

Appointments, etc—R S Spray, for twenty-five years a member of the faculty of the West Virginia University School of Medicine, Morgantown, has retired as professor of bacteriology

H P Rusch, professor of oncology in the University of Wisconsin, has been appointed director of the McArdle Memorial Laboratory for Cancer Research of that university

J G Hoffman, Ph D in physics, has been appointed director of cancer research in the Rockwell Park Memorial Institute, Buffalo

The retirement of Colonel James E Ash, M C, director of the Army Institute of Pathology, Washington, D C, has been announced

H M Weaver, Wayne University College of Medicine, Detroit, has been appointed director of research of the National Foundation for Infantile Paralysis

E L Miloslavich, professor of pathology in Marquette University Medical School, Milwaukee, from 1920 to 1933 and professor of legal medicine in the University of Zagreb, Yugoslavia, from 1933 to 1944, has returned to this country

H S Breyfogle, director of the medicolegal department of St Louis County, in Missouri, has been appointed chief medical examiner for the State of Virginia under the provisions of the act of the legislature establishing a new system of examinations and reports of deaths occurring under unnatural or suspicious circumstances

Deaths—Sophia Getzowa, professor emeritus of pathology at the Hebrew University in Jerusalem, died July 12, 1946, at the age of 74. She was the pioneer pathologist in Palestine, making autopsies in all parts of the country and pathologic examinations for all the hospitals for many years

Winford P Larson, head of the department of bacteriology of the University of Minnesota since 1918, died January 1 at the age of 66

Eugene C Piette, pathologist of the West Suburban Hospital, Oak Park, Ill, died at the age of 54. He was formerly associate professor of pathology in the University of Illinois and for a short time (1918-1920) professor in the University of Kharkov, Ukrainian S S R, Soviet Union

Society News—The Society of American Bacteriologists will hold its forty-seventh annual meeting in Philadelphia, May 12 to 16, 1947

The American Society of Clinical Pathologists will hold its twenty-sixth annual meeting in Atlantic City, N J, June 6 to 8, 1947. The headquarters will be the Ambassador Hotel

The Memphis Society of Pathologists has been formed, with D H Sprunt as chairman

The Pennsylvania Association of Clinical Pathologists has been organized. William P Belk, Philadelphia, is the president, and H F Hunt, Danville, is secretary-treasurer

Hortega Memorial—*Archivos de histologia normal y patologia* dedicates its June 1946 number to the memory of its founder, Pio del Rio Hortega, neuro-histologist, 1882-1945. The number contains notable articles on neurobiologic problems

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